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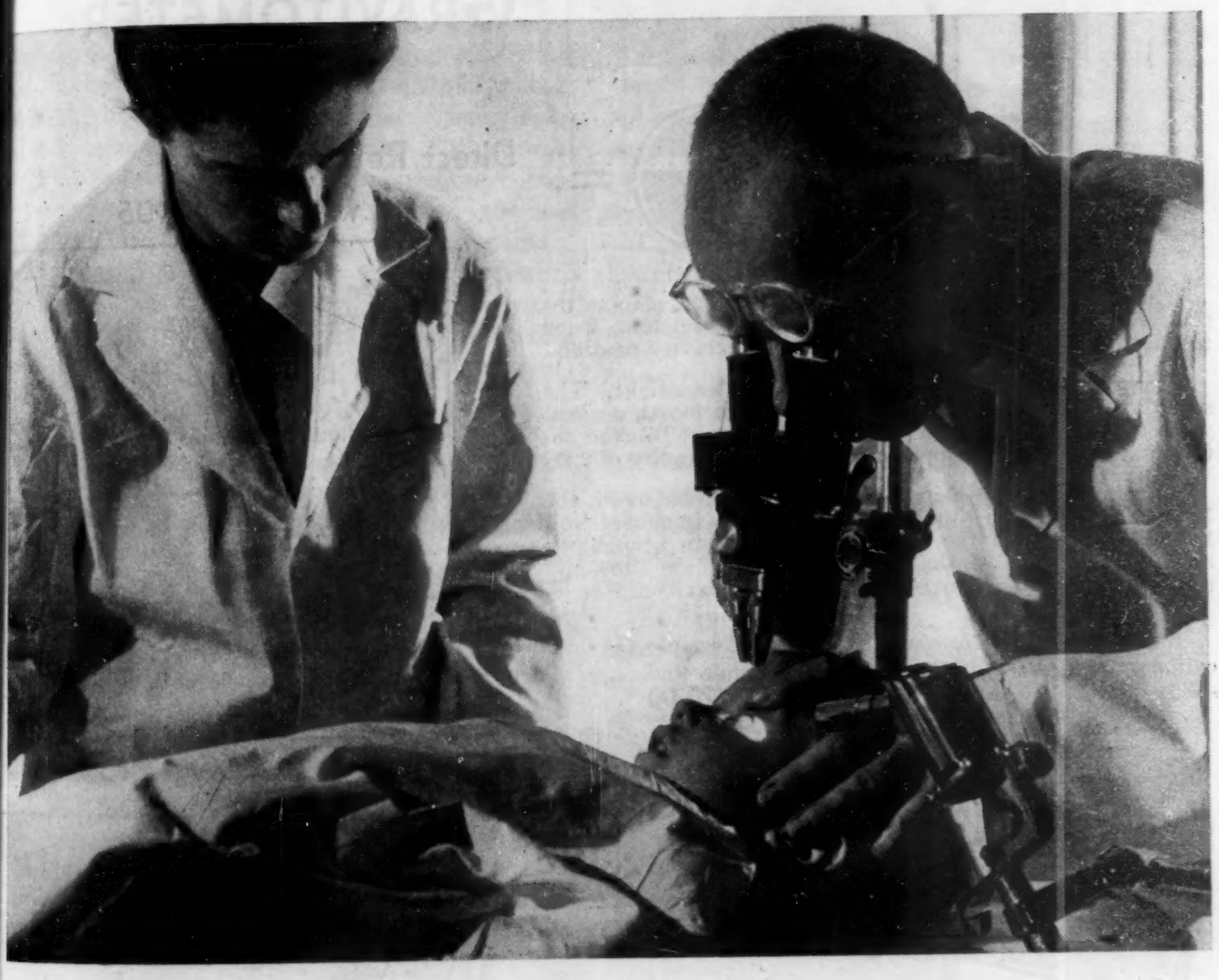

Edward H. Bloch and Louise Warner, who, with Melvin H. Knisely and Theodore S. Eliot, are authors of "Sludged Blood," shown studying the blood and vessels of an unanesthetized, unoperated small child.

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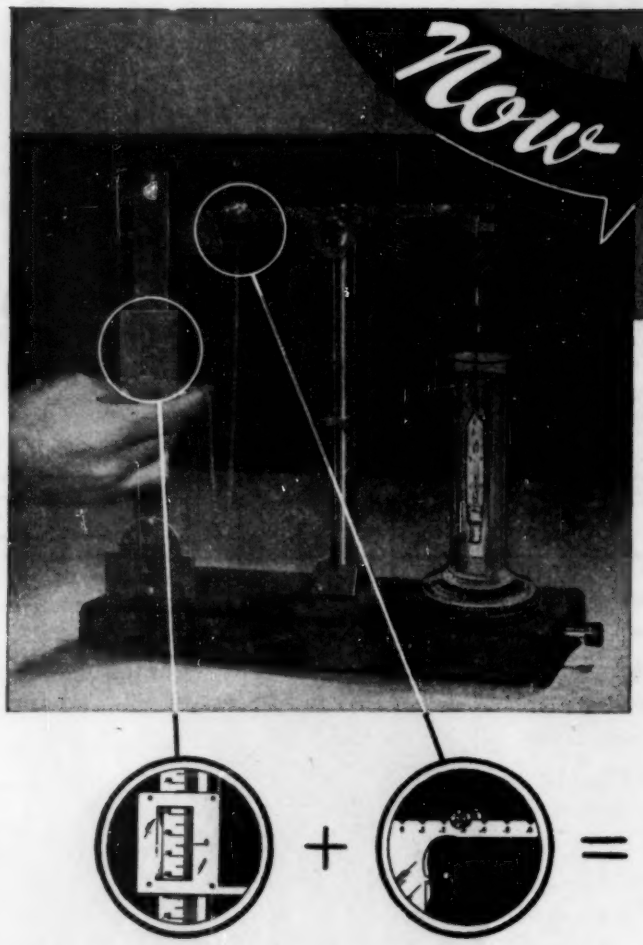
Sludged Blood



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ludged Blood

Melvin H. Knisely, Edward H. Bloch, Theodore S. Eliot, and Louise Warner

Hull Laboratory of Anatomy, The University of Chicago; Department of Zoophysiology, The University of Copenhagen; and *Departments of Anatomy and Preventive Medicine, University of Tennessee Medical School*

THIS PAPER CONSTITUTES A BRIEF INtroduction to a series of observations made mostly with microscopes in living animals and n which leads to a more precise understanding of a riety of mechanisms whereby injuries and diseases mage the human body. It is felt that these observations arify a group of fundamental ideas, explain many old periments, and make the solution of several groups of rrently perplexing problems quite simple. The observaons also permit, and we think necessitate, a subdivision d reclassification, a much-simplified and, for guiding vestigations, a more useful classification of many of the rrently known pathologic mechanisms of the diseases animals and men. Our purpose is to present and define rtain properties of normal blood, blood flow, and vessel alls; to offer evidence that these properties are necessary the normal functioning of the circulatory system; to scribe certain visible responses of the vascular system d/or blood to specific stimuli; to describe certain visible thologic structures and processes; and to define goals w necessary for therapeutics.

The material presented is an outline and summary of 16 ars of observation and experimentation during which o major methods have been used. Living animals—gs, salamanders, mice, rats, guinea pigs, cats, rabbits, gs, and monkeys—have been carefully anesthetized d operated upon, and internal organs such as striated uscles, smooth muscles, gastrointestinal tract linings, rface areas of brain, peripheral nerves, uterus, spleens 2), livers (*27*), omentum, mesenteries, frog kidneys, . exposed. Parts of these organs have then been transuminated with light-conducting fused quartz rods (*23*) d studied with microscopes at 16–600 × magnifications. This was necessary in order to learn about the ucture, dimensions, and natural behavior of the blood l vessels of living internal organs.

Since 1941 binocular dissecting microscopes have been focused on the obliquely illuminated bulbar conjunctival vessels of living, unanesthetized, unoperated animals and men (*25*). When skilfully used, this method causes almost no discomfort to most subjects (*16, 28, 29, 45*) (see cover). Because the blood is thoroughly swirled and mixed in the chambers of the heart and the arch of the aorta, this method permits continuous, careful study of a statistically valid sample of all the subjects' circulating arterial blood (*29*). It also permits study of one set of small human vessels, including both their vasomotor conditions and responses (*3, 11, 14, 30, 38, 49*) and various aspects of the normal and/or pathologic reaction states of their walls (*6*). Magnifications of 32, 48, 64, and 96 × have been used.

The dimensions of many observed structures have been measured or estimated closely with transparent scales mounted in the microscope ocular or recorded in motion pictures.

Healthy, Normal Blood and Vessel Walls

In the internal organs of about 3,500 healthy frogs, 1,100 salamanders (*Amblystoma*), and 500 laboratory mammals anesthetized with pentobarbital sodium and in the bulbar conjunctiva of 50 healthy, unanesthetized medical students and student nurses the following observations have been made:

(1) The circulating red cells not only were not agglutinated but tended to repel each other slightly. In carefully handled tissues red cell rouleaux were not present. The normal red blood cells were not coated with any microscopically visible protein precipitate. The fact that they show no tendency to adhere to each other *in vivo* is evidence that they are not coated with any very thin, transparent, or otherwise invisible sticky precipitate. These observations are in strict agreement with those of many

The work described in this article was aided by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago and from the Winthrop Chemical Company; and by the Tennessee Valley Authority, which initiated this general study of sludged blood through its cooperative program with the University of Tennessee for studies on malaria because patients with many diseases have symptoms similar to those with malaria.

The authors are deeply grateful to Dr. Louis Levy of Memphis for the loan of a universal slit lamp and corneal microscope, without which these studies could not have been initiated; to Roche-Organon, Inc., of Nutley, New Jersey, for generously supplying the heparin used in the study; and to Profs. Robert R. Bensley, Preston Kyes, Frank L. Roberts, Paul Brandt-Rehberg, and August Krogh for suggestions and criticisms which have guided the observations and experiments.

previous investigators (*1*, *2*, *26*, *35*, *36*, *44*). The observations of unagglutinated blood cells in young, healthy human beings are particularly valuable as a strict control for animal experiments since these human beings had not been subjected to fright, anesthetics, or operations.

(2) No white cells or erythrocytes stuck to the inner surfaces of the walls of small vessels. The inner surfaces of the linings of normal small vessels were smooth and clean (*6*, *35*, *36*).

(3) The flow of the unagglutinated blood was laminar or "streamlined." In small arteries and veins the blood cells were in an axial stream and around them was a peripheral concentric layer of plasma (*30*, *44*, *49*, *50*). The cells of this stream were arranged in concentric laminae, the center one passing along most rapidly and each additional layer passing more slowly than the one inside it. The wall of each lamina of this system consisted of unagglutinated blood cells; each layer was exactly one red cell thick (cinema recorded). This arrangement of unagglutinated blood cells in fluid plasma is a necessary part of the highest degree of good health, as the next and succeeding paragraphs show.

The rates of flow of blood through each tissue of each organ of the body set the maximum rate at which the cells of that tissue can receive blood-borne materials. For this discussion the most important of these is oxygen: it is necessary to all cells of the body, it is not stored in the body, and even slight local oxygen deficits are known to begin to upset many factors of physiology. The rates of flow of blood through each tissue are precisely controlled by the narrowest and/or most powerfully contractile vessels in the tissue, the arterioles, terminal arterioles, contractile sphincters, etc. In healthy animals the diameters of these vessels may remain nearly constant under uniform experimental conditions or may change from moment to moment as parts of *necessary* physiological adjustments of the rates of supply of oxygen and glucose to tissues, the rates of removal of waste materials and heat from tissues, or the rates of delivery of blood to some organ (e.g. kidney, spleen, skin, liver) which performs some special indispensable function upon the blood it receives (*27*).

The diameter of each and every small vessel is determined at any time by the balance between the outward blood pressure and the current degree of contraction of the smooth muscle and/or other contractile elements of the vessel wall, plus, in some tissues such as muscle, the varying pressure of surrounding structures. The small vessels are not capable of infinite physiological dilatation, for, if the contractile elements are maximally relaxed, the diameters are then determined by the length, elasticity, and elastic limit of the connective tissue fibers which form a close-meshed basketwork surrounding every vessel.

During health the physiological changes in the diameter of each and every set of small vessels are precisely controlled by changes in the degree of contraction of the contractile elements of the vessel wall, in response to changes in the equilibrium between "constrictor and dilator" substances which can reach the vessel wall by three paths—from blood flowing through the vessel, from cells of the particular tissue or organ around the vessel, and from nerve endings in, upon, or near the vessel wall. The responses of the contractile elements are also continuously affected by temperature changes in the tissues around vessels and in the entering arterial blood. Many of these reaction patterns are exceedingly complex (*22*, *27*); all are necessary parts of the internal adjustments of normal good health. The previously described arrangements of the semisolid blood cells suspended in the moving plasma cause the minimal internal friction in the columns of blood in arteries and veins, thus permitting the available pressure drop along each open vessel to cause the most rapid possible rate of blood flow through that vessel. As a direct, and most important, consequence of this, every homeostatic dilatation of any set of small vessels is immediately followed by the maximum hydrodynamically possible increase in the rate of flow of blood through that set of vessels and the maximum possible increase in the rate of supply of oxygen, rate of removal of wastes, etc.

(4) The small, normal vessels of most tissues and organs did not leak appreciable amounts of fluid for (a) there was no visible hemoconcentration occurring (*30*, p. 14); (b) in the mesenteries and omentum of animals the fat cells outside vessels were tight together, not pushed and held apart by escaping fluid; and (c) in human beings the bulbar conjunctiva was not forced up and held away from the sclera, whereas this does occur and can easily be seen with stereoscopic microscopes in human beings at times when visible continuous hemoconcentration is occurring in leaking bulbar conjunctival vessels. The complicated permeability phases of spleen and liver vessel walls are described elsewhere (*22*, *27*).

(5) The blood flowed so rapidly in most arterioles and venules which were from 60 to 120 μ in diameter that individual red cells could not be seen. This is a crude but accurate and useful criterion for adequate rates of blood flow through most open-tissue capillaries. If the rates of flow of oxygen-saturated arterial blood are fast enough so that individual unagglutinated blood cells cannot be seen in vessels of this size in normal animals and men, high magnifications show that no visible hemoconcentration is taking place in capillaries, *i.e.* almost no blood fluid is being lost through the walls of the tissue capillaries.

(6) The shapes and dimensions of the vessels of the peripheral vascular beds constitute a perpetual bottleneck in the circulatory system. All the vessels which carry blood toward tissues are long, narrow, slowly tapering truncated cones. When arterioles branch, each branch has a narrower lumen than that of the parent stem. The capillaries or sinusoids which carry blood through tissues are approximately cylinders, and the veins are slowly widening cones again. Arteriovenous anastomoses have been

found in some tissues, but these have small total surface areas; if oxygen, glucose, and other blood-borne anabolites are to be distributed effectively to the cells of a tissue, the blood must flow through the capillaries or sinusoids of that tissue.

We have found that almost every whole arteriole-to-capillary or sinusoid-to-venule pathway can contract tightly shut throughout its length, making its internal diameter zero and thus forcibly resisting the entrance of blood, and that individual parts or all its parts can dilate maximally (*27*). As noted, the pathway is not capable of infinite physiological dilatation because of the elastic limit provided by the basketwork of connective tissue fibers surrounding each arteriole. Forcibly dilated capillaries begin to leak proteins and do not retain blood plasma.

In the internal organs of the mammals we have examined, the open arteriole tips were, for the most part, narrow enough so that every red and white cell passing through was forcibly distorted—usually pinched and elongated or folded. This is also clearly shown in frogs in a motion picture prepared by Fulton and Lutz (*14*). The capillaries through which blood is flowing normally vary from a little less than once to as much as two to two and a half times the diameters of the passing red cells. Most true capillaries can dilate without losing tonus, weakening and sacculating to a little more, but not much more, than two or two and a half times the diameters of the animal's own red cells.

These statements are true of the arterioles and capillaries of the bulbar conjunctiva of human beings, whose red cells are usually a little less than 8 μ in diameter, and almost certainly true of all the arterioles and capillaries of most human organs during life. Thus, as is well known but not always remembered, under all conditions of health and disease the arterioles and capillaries are a perpetual bottleneck in the vascular system.

Normal, unagglutinated, circulating blood and normal vessel walls of monkeys have been recorded in colored motion pictures (see Reel 1 of the motion picture, "Knowlesi Malaria in Monkeys," *29*).

One other aspect of normal circulation, although important, has not been studied in living human beings. In living frog and rhesus monkey livers the normal naked red cells slide and bump along the surfaces of the highly phagocytic cells which line the hepatic sinusoids, but *no normal naked red cell has ever been observed to be ingested.* These phagocytes continually "ignore" normal naked erythrocytes (*27*).

The factors of normal blood, blood flow, vessel walls, and vascular behavior listed above set the stage for recognition, understanding, and evaluation of, and planning new therapy for, a variety of factors of circulatory pathology. Once any anatomical, physiological, behavioristic, or chemical aspect of normal healthy animals or human beings can be sharply recognized and clearly defined, all detectable deviations from that aspect of the normal can also be recognized and defined. Once a *kind* of deviation can be defined, the detectable degrees of that kind of deviation can be arranged in scales whose extremes are the minimal perceptible deviation from normal and the maximal degree of that deviation which can exist and still have life continue. The return of each kind of pathologic deviation to the previously defined healthy normal then becomes an immediate and continuing goal of rational experimental and applied therapeutics.

The Circulating Blood of Human Patients

In about 600 unanesthetized human patients diagnosed by practicing physicians as having a wide variety of pathologic conditions and diseases, we have seen the blood cells agglutinated into masses (not rouleaux); this changed the blood from its normal, relatively fluid state, to a circulating sludge. The variety of the diagnoses is attested to by the following partial list, which includes the number of patients seen with each[1]: bronchiectasis, Buerger's disease, large acute burn, diphtheria, eclampsia, acute streptococcal endocarditis, gonorrheal salpingitis, granuloma inguinale, hysteria, chronic lymphatic leukemia, uncomplicated vivax malaria, multiple myeloma, normal uncomplicated pregnancy, thyrotoxicosis, typhus fever, whooping cough, traumatic shock without external hemorrhage (1 each); bronchitis, lung abscess (nontuberculous), malignant hypertension, measles, myelogenous leukemia, multiple sclerosis, subacute bacterial endocarditis, trichinosis, tularemia, Weil's disease[2] (2 each); nephritis, portal cirrhosis, smallpox, thrombopenic purpura, typhoid fever, varicose leg ulcers (3 each); common cold (4); meningococcic meningitis, neoplasms of testis, colon, esophagus, pancreas, and one of unknown primary origin with multiple metastasis (1 each); traumatic shock complicated by hemorrhage and acute alcoholism (automobile accident cases) (5 each); scarlet fever, sickle cell anemia (6 each); acute arsenical reactions (7); gonorrheal arthritis, syphilis of the central nervous system (8 each); acute rheumatic fever (9); central nervous system syphilis under treatment with falciparum malaria (11), with vivax malaria (10), with quartan malaria (18); undulant fever (13); pneumococcic pneumonia (18); acute alcoholism

[1] The patients in these lists were studied at the John Gaston Hospital, Memphis, Tennessee, where the investigation was initiated, the Iroquois County Hospital at Watseka, Illinois, where the undulant fever patients were available, and the Municipal Contagious Disease Hospital, the Municipal Tuberculosis Sanitarium, Michael Reese Hospital, Billings Hospital, and the Chicago Lying-in Hospital in Chicago. The administrative officials and staffs of these hospitals have all been most generous and cooperative in creating opportunities for us to study their patients. Many physicians have taken considerable time to be certain that we studied very carefully diagnosed patients. Among these are L. W. Diggs of Memphis, who provided the sickle cell anemia patients; Earl Roberts of Watseka, the undulant fever patients; Gilbert Levi of Memphis and Archibald Hoyne of Chicago, patients with highly infectious diseases; Henry C. Sweany, patients with tuberculosis; and Charles Dunham of the University of Chicago, the arthritis patients. To these and many others we are deeply grateful.

[2] The agglutinated circulating blood of one Weil's disease patient was photographed on Eastman Supersensitive XX 16-mm. movie film.

(20); acute anterior poliomyelitis (21); heart disease (luetic, 5; arteriosclerotic, 15; pericarditis, etiology unknown, 2; rheumatic, (6); pulmonary tuberculosis (58); and rheumatoid arthritis (125).

Odell, Aragon, and Pottinger, using a modification of our apparatus, have studied the circulating blood of 21 women with normal uncomplicated pregnancies and found sludged blood in 12. Of 23 women with various pathologic complications of pregnancy, 22 had sludged blood.

Vessel Walls of Human Patients

Almost all the known types of vessel wall pathology visible *in vivo* have been seen in this study of human patients: arterioles both temporarily and permanently plugged with masses of sludge, and, in some, short, spindle-shaped bulges (aneurysms) of arterioles (*45*).

The Clarks (*6*) arranged a series of pathologic reaction states of capillaries and small veins in terms of increasing degrees of response to increasing degrees of experimental injury, which permits classification of many of our observations in terms of the damage already done the vascular system rather than in terms of the patient's diagnosis. Following their classification, we have observed (a) white cells rolling along vessel linings; (b) white cells sticking, sometimes for hours; (c) white cells in layers (particularly prominent in the 3 leukemic patients, who also had circulating masses of agglutinated white cells); (d) weakened, dilated, bulged, sacculated, rapidly leaking capillaries and postcapillary venules; (According to the Clarks, and our experience is in agreement, the above stages of individual capillary and small vein wall injury are reversible to normal.) (e) true stasis, *i.e.* vessels which had been leaking rapidly, packed with red cells and/or masses of agglutinated red cells; (f) scattered red cells; and (g) small hemorrhages outside vessels which are evidences of high previous porosity or rupture of vessel walls.

Further, in many patients large areas of the conjunctival vascular system, arterioles, capillaries, and veins had been so tightly constricted that no red cells were visible or passed through, sometimes for hours. This can frequently be seen in white patients who have marked general pallor and was conspicuous in every one of 10 consecutive patients who had far-advanced pulmonary tuberculosis.

The arterial blood pressure of many of these patients was taken. In all those listed it was within normal range or above. Further, these did not show evidences of increased venous pressure. Hence, the slow passage of this sludged blood through open vessels was directly due to the sizes and rigidities of the masses, not to increased venous pressure, failing venous return, or cardiac failure.

These patients have been studied as a part of a general survey designed to find the extent of the phenomenon of intravascular agglutination of the blood. This list includes all patients in similar lists published previously (*25*).

The patients in the lists are unselected—that is, no attempts have been made to keep from studying patients with any particular disease, and every patient seen who had any particular diagnosis is listed. Sludged blood has been observed in men and women, white and colored people, and persons of all ages.

Thus far, completely unagglutinated blood has been found only in strictly healthy animals and men. Mild degrees of intravascular agglutination have been seen in many laboratory workers, students, and colleagues in the Chicago area, where sinusitis of various degrees of severity and other afflictions of the upper respiratory system are endemic. The more severe degrees of intravascular agglutination have been exhibited only in animals during controlled experiments and in persons who were sufficiently ill to have placed themselves under the care of physicians. No severely ill person has yet been seen who did not have intravascular agglutination of the blood and visibly pathologic vessel walls. The survey is, of course, still in progress and should be extended as rapidly as skilled observers can be trained and opportunities made available. The ultimate survey obviously should include all the diseases and pathologic conditions of other vertebrates, including those of food fish, birds and poultry, household pets, laboratory animals, and all the mammals on which people depend for food, clothing, transportation, and the commercial production of the vaccines and therapeutic sera, as well as all the other diseases of humans.

Since 1852, when Coccius (*8*) published microscopic observations of agglutinated blood in living human patients, sludged blood has been seen or demonstrated in living animals and in human patients and some of its results observed *in vitro* or in histological sections by many investigators (*9, 12, 13, 19, 21, 23–27, 33, 35, 36, 39, 45, 46, 52*). Since Landsteiner's (*32*) demonstration of the human blood groups, microscopic study of phenomena associated with, or resulting in, agglutination of blood cells on slides and macroscopic studies in test tubes have been in continuous use in research laboratories and are now a standard part of the blood-matching techniques in all the hospitals of the world. As a result of such studies, there is an immense immunological literature describing and analyzing *in vitro* agglutinations (*48*).

Further, as a part of the classical investigations by which Robin Fahraeus (*9*) initiated the well-known erythrocyte sedimentation-rate test, he and his student, Ploman, clearly demonstrated by three separate methods that there is increased aggregation of circulating red cells within living human patients. Fahraeus' work is sharply relevant to subsequent discussions in this paper, for it forms a major connecting link between (a) the humoral pathology of the ancients, which dominated all of medicine from Hippocrates to the beginning of the cellular pathology of Virchow, (b) a large and continually growing body of knowledge about the composition and physical behavior of blood drawn from different classes of patients

nd (c) the whole subject of intravascular agglutination f the circulating blood.

By means of *in vitro* studies Fahraeus showed that the ncreased sedimentation rates of the red cells of blood rawn from human patients were due to alterations in the hemistry of the plasma rather than to detectable changes n the blood cells themselves, and he made the first direct ontributions to the understanding of the chemical hanges in plasma which are associated with, and can nitiate, increased *in vitro* sedimentation rates.

After a great many *in vivo* observations, Fahraeus deduced and carefully pointed out that (a) "the gas exchange between the corpuscles on the one hand, and the tissues and the alveolar air on the other, takes place via the plasma. As the aggregation of the corpuscles reduces the surface between the corpuscles and the plasma we must a priori conclude that it affects the gas exchange of the corpuscles in an unfavorable manner"; (b) agglutinated red cell masses could be expected to act as minute embolae, *i.e.* be carried into and plug small arterioles and capillaries, and multiple minute embolae are found at autopsy following many diseases; (c) reduced suspension stability of the blood must "play an important part in the genesis of thrombi—as well as concerning the red parts of the mixed great thrombi in the larger vessels as with regard to certain kinds of hyaline thrombi of the capillaries, which according to the statements of the literature are composed of fused red corpuscles and which especially characterize the changes of the bodily organs in eclampsia."

As a result of Fahraeus' *in vitro* investigations, the red cell sedimentation-rate test has become a standard part of clinical medicine and is in use in all hospitals and by nearly all practicing physicians throughout the world; in consequence, significant alterations of the rates of settling of erythrocytes have been found in blood from patients having many different diagnoses.

There has, however, been no rigorous, systematic search for all the biological, physical, and chemical etiologic agents capable of initiating intravascular agglutination of the blood or for all the chains of specific chemical and/or immunological reactions which can and do initiate *in vivo* agglutination; nor have there been systematic attempts to correlate observed and measured effects of intravascular agglutination with the signs and symptoms presented by sick people or animals, to determine the role of intravascular agglutination in the development of the lesions observed at autopsy, or to determine the kinds, degrees, and rates of damage this set of mechanisms can do to living animals and men.

How Sludged Blood Damages the Body

The concepts presented below were developed over a 7-year period, partly by comparing many observations of the agglutinated blood and damaged leaking vessel walls of diagnosed sick persons with those of the unagglutinated blood and intact vessel walls of healthy animals and men, partly by almost painless experiments upon unanesthetized, unoperated, healthy adult human volunteers, but most of all by completely indispensable, sometimes almost daily, laboratory experiments upon carefully anesthetized amphibians and mammals, including cats, dogs, and rhesus monkeys.

I. The resistance of sludged blood to its own passage through the bottlenecks of the circulatory system forcibly reduces the rates of blood flow through all the open vessels of the body.

II. Agglutinated red cells are ingested and destroyed in the phagocytic cells of liver and spleen.

III. There is settling and sedimenting of masses of agglutinated blood cells out of the moving blood plasma during life.

IV. Various degrees of reduction in circulating blood volume caused by I and II, above, initiate intermittent, prolonged, controlled shutting off of the arterioles of a selected series of tissues and organs.

Each of these categories has its own set of associated phenomena and known and probable consequences.

There are many different kinds of blood sludges, and the known and probable kinds of injury, degrees of these, and the rates at which any particular sludge damages an animal or man, depend to a great extent upon two sets of factors: (a) the as yet largely unknown chemical composition of the material or materials which hold blood cells together in wads, and (b) the easily observable physical characteristics of the masses of which that particular circulating sludge is composed.

A very simple sludge is one in which all the red cells are in masses, all masses are approximately the same size when they are in vessels which do not compress them, every mass is sufficiently cohesive internally so that it does not break up as it passes through the peripheral arteriole-capillary-venule bottlenecks, and all masses passing through any one bottleneck are compressed and elongated to an equal degree. By definition we are calling these masses which do not break up as they pass through the bottlenecks the "basic masses" of a sludge, usually abbreviated to "the basics." If we call the resistance of a mass to distortion the "rigidity" of the mass, then the sludge delineated above would be described as homogeneous—one with no free red cells, all basics having the same size and rigidity.

In addition, there are mixed sludges of many kinds: those in which free, unagglutinated red cells are also present; those in which several sizes of basic masses are simultaneously present; those in which the largest basic masses continually act as temporary embolae, *i.e.* temporarily plug each and every arteriole they enter. The concentration of such large masses per cubic centimeter of blood determines, of course, the frequency with which every terminal arteriole of the body is temporarily plugged. The time during which any arteriole is temporar-

ily plugged, and the tissue it supplies receives no blood, often depends upon the rate at which the mass distorts to a spindle narrow enough to pass through the bottleneck, *i.e.* probably on the thixotropic properties of the mass (*40*). In addition, the plugged arteriole sometimes goes through slow, rhythmical contractions which may or may not compress the mass to a spindle.

There are sludges in which most basics are small, but larger masses come along at short or longer intervals, each permanently plugging whichever arteriole it enters. The damage done by these permanent plugs depends, of course, upon their numbers, their concentrations in blood at any one time, the particular tissues they happen to enter, and the summations of the lengths of time such masses enter small, isolated parts of organs. Such masses can, in relatively low concentrations, be utterly devastating over a period of a few weeks, months, or years. One young woman referred to us from a psychiatric division because she had a slightly elevated red cell sedimentation rate in addition to her "functional" psychological disturbances had 8 small permanent plugs in the terminal arterioles of the bulbar conjunctiva of one side of one eye. They were in different stages of the familiar hemoglobin chemical disintegration color series. Every once in a while a slightly smaller mass came along, temporarily plugged a vessel, and passed on again. Two weeks later the patient had three more similar permanent embolae of terminal conjunctival arterioles. From the branching pattern of the aorta and the great arteries to the head, and the fact that the blood going to the eye comes directly from these, one cannot doubt that this woman's whole central nervous system was slowly showered with permanent plugs, each of which destroyed a small volume of irreplaceable nerve cells. When one considers the parallelism between the known effects on normal persons of breathing slowly decreasing concentrations of oxygen (cerebral effects of anoxia), the slightly to greatly increased irritability, the euphoric tendency to laugh uproariously at meaningless trivia, the dull-witted phases, the compulsive behavior at times, and the comatose condition as the anoxia approaches the lethal stage, and similar phases of some of the symptom complexes studied in mental hospitals, it is obvious that several groups of psychiatric patients now need to be studied to determine the role which sludge is playing or has played in their pathologic physiology. Osler himself pointed out that psychic disturbances can and do follow infectious diseases (*43*).

There are sludges composed of masses which repel each other slightly when they are in venules and sludges in which the basic masses come together slowly or rapidly, sometimes from relatively long distances, whenever the vein blood slows down or temporarily stops. These agglomerates we have been calling "charge aggregates," because the basics obviously exert force on each other from a distance.

There are sludges in which the masses, both basics and aggregates, are very sticky and hold together with various degrees of tenacity, as can be seen where streams of blood come together in small veins, setting up "couples" of force which produce torques tending to pull such masses apart. There are also sludges in which the masses seem coated with glassy, hard materials and display no tendency to adhere to each other.

There are sludges in which the masses are bright red, in which case it must be presumed that little, if any, material is between and around the red cells holding them together; and there are still other sludges in which the masses are pale pink and, when they bump each other, behave as though a transparent invisible layer was around the red cells in each mass extending beyond the red cells.

I. All the sludges in which the masses are large and rigid enough to resist passage through the bottlenecks of the circulating system forcibly cause reduced rates of flow through all open vessels and thereby continually act toward reducing the rates of supply of oxygen to endothelium, initiating and maintaining endothelial anoxia and its consequent inevitable, well-known permeability of the endothelium to blood plasma proteins (*28–31*). This initiates continuous loss of fluid from, and continuous hemoconcentration of, the passing blood and, if the lost fluid is not rapidly removed by lymphatics, edema of the surrounding tissues. Monkeys with the stiff Stage III Knowlesi malaria sludge often die mainly from this series of effects. This has been carefully recorded in motion pictures (*29*). When these effects of forcibly reduced flow rates are of sufficient magnitude in the vessels of the conjunctiva of ambulatory patients, the patients almost always have grossly visible edema of the feet and ankles when they stand or walk for a time. This is particularly noticeable in patients with rheumatoid arthritis.

In each patient in which these sludge factors are present, they may, as far as we now know, be expected to operate in addition to any other known factors which may be increasing the rates of fluid loss through endothelium. No human patient whom we have studied who had grossly visible edema during bed rest has failed to have large semirigid masses, visibly slowed rates of blood flow through the conjunctival vessels, different degrees of plasma loss through the walls of these vessels and of microscopically visible hemoconcentration, conjunctival edema, etc. As is well known, many human patients have various degrees of unexplainable edema, particularly of the ankles when ambulant, continually or for shorter or longer periods, for years. These can now be examined for sludges and their detectable effects.

In view of the recent excellent studies of O'Neill (*42*) on the anatomy and physiology of the small vessels in the walls of large vessels (the vasa vasorum) which are necessary to nourish the tissues of the larger ones, and the role which stopping the blood flow through these small vessels plays in the damage of the lining of the large vessel, it is

necessary to examine the possible roles of the sludges, which must forcibly decrease the rates of flow of blood through these small vasa vasorum, in initiating those pathologic histological changes found in human beings with arteriosclerotic disease, etc.

When all the masses of a relatively homogeneous sludge are sufficiently large and rigid, they resist passage through the peripheral vascular beds enough to cause death in a relatively short time, sometimes in experimental animals within 3 to 6 or, at most, 12 hours. Many of the details of this kind of death in monkeys have been carefully described and recorded in motion pictures taken through microscopes (*29*). Untreated, unanesthetized, unoperated monkeys with stiffly agglutinated Stage III Knowlesi malaria blood go into a slowly deepening comatose condition ending in deep coma before death. The heavily sludged blood of these animals could be studied after they were in coma in the inner surface of the gently reflected eyelid. In a number of deeply comatose human hospital patients examined by us the blood has also been agglutinated into large, pasty, sticky wads moving very slowly through rapidly leaking vessels (*7*). One such patient had a slightly elevated arterial blood pressure. Hence, the slow passage of his agglutinated blood was due to its mechanical condition rather than to failing venous return or cardiac failure. Heavily sludged blood has also been found in accidentally ill, unanesthetized, comatose rabbits and cats. Hence, it is now necessary to examine adequate numbers of cases of all the diseases of lower vertebrates, as well as of men, which can have a comatose condition as a temporary or terminal part of their pathologic physiology. Whenever large, rigid, slowly moving masses are found, drugs can easily be tested to find some which will cause or permit disintegration of the masses (*29*). When sludges are not present, experiments can be devised to find other, as yet perhaps unknown, causes of comatose conditions.

II. The rapid ingestion of masses of agglutinated blood cells by the phagocytes of the spleen and liver probably is a major factor in the initiation and maintenance of many classes of human anemias. This subject is now ready for rigorous investigation in the postoperative and postburn and so-called convalescent anemias. This factor may also be operating in many of the more specifically named and diagnosed anemias.

The subject of selective phagocytosis of particles from the circulating blood has been under rigorous investigation in our laboratory for some time (*27*). In frogs injected with India ink, each injected particle immediately receives a coating of a visible sticky material, probably protein. Monkeys in Stage II of Knowlesi malaria have a sticky coating between and around those red cells which contain malaria parasites. This coating material holds the parasitized red cells together in small, sticky clumps. In both the above situations the coating material, together with the ink or the parasitized red cells within it, has been microscopically observed to be instantly ingested upon contact with any one of the phagocytic cells lining the sinusoids of the liver (*27*), while uncoated, normal, naked, unagglutinated red cells have been completely ignored by these stationary phagocytes. In one monkey in Stage II of Knowlesi malaria, the parasite count fell from 46 to 23 per cent in three hours, which accessory experiments have shown can occur only by selective phagocytosis of coated clumps of parasitized red cells from the circulating blood by the phagocytes of the spleen, liver, or bone marrow. Depending upon the numbers of new red cells one assumes that this monkey might have made during that three-hour period, this experiment may be interpreted as demonstrating that the phagocytes of this monkey ingested and destroyed from one-fourth to one-third of all the animal's circulating red cells in three hours—the maximum rate of phagocytosis of coated blood cells thus far observed. Present experimental evidence does not permit quantitative estimates of the rates at which the phagocytes of these three organs can selectively remove clumps of coated, or perhaps even uncoated, agglutinated red cells from the blood stream. There is the further problem of attempting to find which kinds of masses are ingestible, which not, and what factors limit the rates of ingestion during any particular set of pathologic processes (*27*).

Whenever these phagocytes ingest protein-coated red cell masses, each ingestion must remove a finite amount of protein from the circulating blood. The rapid ingestion of large numbers of red cell masses, each jacketed with finite amounts of protein, may be a major factor in the protein depletions which occur in many human hospital patients (*27*). If such jacketed masses contained specific immune proteins when phagocytized, this conceivably could waste large amounts of these exceedingly precious substances (*4*). If viruses attach to red cells *in vivo*, as Hirst (*17*) has found them to do *in vitro*, the ingestion of coated "red cell plus virus complexes" could be an important part of the defense against these organisms.

One limiting factor which would seem to prevent the phagocytosis of agglutinated blood cells from running an animal or man into hemorrhagic shock is the fact that the phagocytes which remove these masses from the blood are in the blood reservoirs, spleen and liver, and that with progressively decreasing blood volume the vasomotor system empties these organs and then sharply reduces the blood flow into them (*27*).

III. In rhesus monkeys with malaria and in traumatized frogs the masses of agglutinated red cells tend to settle out of the columns of moving blood mostly in those vessels which are horizontal or nearly so and particularly in those nearly horizontal vessels in which the blood is running slightly uphill. This *in vivo* sedimentation of blood cell masses is now being actively investigated because the sedimentation and subsequent cementing together of large numbers of stationary agglutinated masses probably is a major factor in the formation of many venous thrombi (cf. *5*, *10*, *18*, *20*, *34*, *37*, *41*, *47*, *52*, *53*).

Frequently in human beings after operations or during infectious diseases (*5*) large venous thrombi form in the big veins of the legs. These may be of the diameter of a person's thumb and several inches long or more. Later, parts or all of such big masses break loose, pass up into the right heart, and enter the big arteries going to the lungs. Small masses plug small pulmonary arteries, causing pulmonary infarcts of various sizes. Large masses may fill the right heart or completely plug the pulmonary artery, causing instant death. This is an even more common cause of human death than had been suspected until recently (*5*).

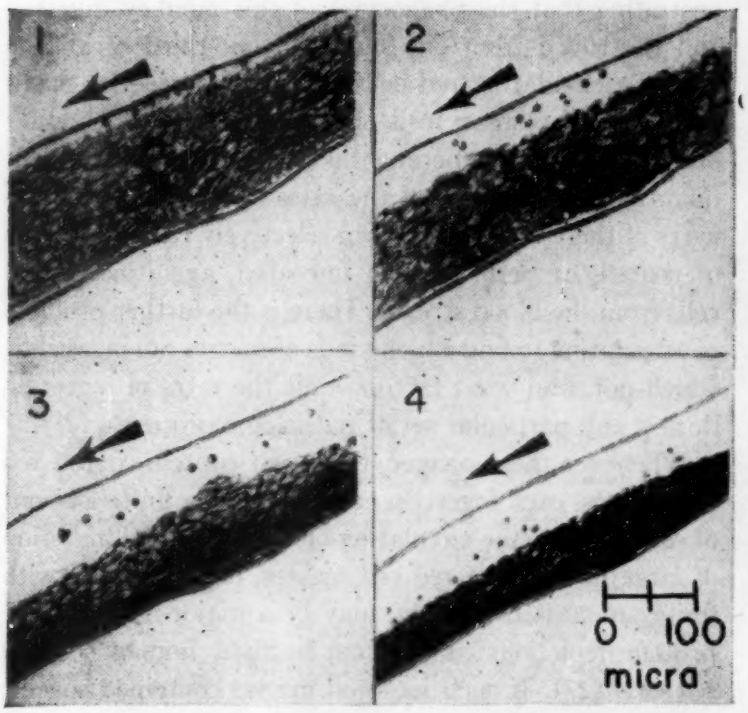

FIG. 1. Small frog mesenteric vein viewed from the side as sticky red cells and red cell aggregates slowly settle in moving blood. In 4 the masses have become cemented tightly together, making a small, lightly attached thrombus.

By suspending a fan-shaped loop of intestinal mesentery of a frog in the vertical plane and sending a beam of light through the membrane into the objective of a horizontal microscope, it is possible to view the side of all the vessels in the mesentery and watch the effects of gravity on the masses of agglutinated cells in the moving blood of all these vessels. The operation must be done without losing any blood from the frog (*27*). The trauma caused by the laparotomy initiates formation of adequate amounts of sludge for study in this preparation. The masses of sludge passing through the vessels begin to settle in every vessel which is horizontal or nearly horizontal when certain very definite physiological and physical conditions are provided. The masses must be heavier than the fluid, and the rate of flow in the nearly horizontal vessels must become slow enough so that masses cannot be carried in suspension. The resistance of the sludge to passage through the bottlenecks frequently provides sufficient reduction in flow rates. There is probably a critical speed for each size of vessel above which the red cell masses are rolled over and over and up into the axial stream; below this critical rate of flow the masses settle, the largest first. Once this process begins, the bottom layer of masses stays relatively still, and the upper layers may roll along rather slowly. Thus, the effective cross-sectional area of the vessel changes from something which approximates a circle to something which approximates the upper two-thirds or one-half of a circle. In some preparations in frogs, the masses of settled agglutinated red cells remained free from each other even when packed tightly together for an hour or two. In other preparations those masses packed together in the bottom of a vessel slowly became cemented together into long, gelatinous masses (Fig. 1). The masses settle more rapidly if the animal has a low rather than a high red cell count. This is in strict agreement with the fact that blood cell masses sediment very rapidly *in vitro* in blood with low red cell counts. The rates of flow of blood through the frog mesentery vessels can be decreased by giving drugs which decrease the animal's rate of heart beat. After a hemorrhage a frog shuts off the arterioles in many tissues (*27*). The rates of flow in small veins are thereby sharply decreased, in addition to the slowed flow already forcibly produced by the resistance of the sludge to its own passage through the bottlenecks. In animals in this condition the rates of settling of agglutinated masses in horizontal vessels are the fastest we have seen.

From the simple experiments described above and simple physical principles, it seems necessary to suspect that in many, and perhaps all, pathologic conditions in which blood cells are agglutinated, the masses tend to settle out of the moving blood stream and be deposited upon the bottom of almost every horizontal vessel of the body. Whether these masses stick to each other should depend upon their charge, degrees of stickiness, etc. Whether the whole long mass sticks to the vessel wall, or a layer of white cells on the vessel wall, must depend upon their current surface characteristics (*6*, *42*).

Such simple experiments with thin vertical tissue and horizontal microscope, and variations of them, which can be easily made using mammals with defined pathologic conditions, should make it an easy matter to determine (a) all the circumstances under which the masses will settle in the circulating blood; (b) the conditions under which they will become cemented together into a thrombus; and (c) the effects of blood transfusions and drugs toward preventing such settlings and thrombus formations in the horizontal vessels.

IV. Many hospital patients have had large areas of conjunctival vessels tightly closed for long periods. Some have shown other evidences of prolonged vessel spasms, e.g. cold lower legs and feet of far-advanced tuberculosis patients. One would expect that the leakage of vessels and phagocytosis of blood cell masses should reduce the blood volume. This, of course, could not be determined by red cell counts or the use of dye methods. Dye methods cannot be expected to have meaning in patients whose vessels are leaking the proteins on which the dyes attach, for the

dye dilutes into tissue fluid and lymph as well as into blood. In order to see if controlled hemorrhage does initiate visible spasms of the conjunctival vessels, the blood and vessels of 34 blood donors, unanesthetized, unoperated relatives and friends of hospital patients, were studied before, during, and for 30 to 60 minutes immediately after each donated blood at the hospital blood bank. The experiment was made originally to identify and separate the mechanisms of traumatic shock, trauma without hemorrhage, in which thick sludge has always been present (*25*, *28*) from detectable effects of hemorrhage without trauma (*38*). This method permits study of the vascular responses of healthy persons following almost no trauma (one stick with a large needle) but with large, controlled hemorrhage.

When a donor had lost not less than 300 and not more than 500 cc. of blood, first one set and then another of the blood pathways made up of arteries, arterioles, capillaries, postcapillary venules, and venules of the bulbar conjunctiva began to contract tightly shut, ejecting all their contents into the venous system. By the time most persons had lost 500 cc. of blood, large areas of the conjunctiva were completely white, only a few small, open pathways remaining. One muscular man was bled 800 cc., whereupon he began to sweat profusely—a common sign of an approaching "shock" condition. He had almost no visible vessels or blood left in the conjunctiva. At no time within the first hour did any intravascular agglutination appear in response to this degree of hemorrhage (*25*, *28*). In most persons large areas of the vascular system, including the capillaries, remained tightly shut for at least 30 minutes.

This series indicates that decreased blood volume is *alone* a sufficient stimulus, when inhibitions and counterstimuli are not present, to initiate prolonged contractions of a variety of small human vessels and that the tissues they nourish may, in consequence, go without oxygen, glucose, etc. for considerable periods (*3*, *11*, *15*, *27*, *30*, *38*, *49*, *51*).

When one considers the numbers of already observed pathologic conditions during which a sludge was present, the fact that many sludges could be expected to do at least small amounts of permanent damage to the body, the fact that these damages must be cumulative over a long period, and the pitiful mental incompetence of the prematurely senile and of many aged persons, it becomes obvious that we now need to begin to determine carefully how all the damages done to the body by sludges can summate as parts of the aging processes. In which different and overlapping combinations can the anatomical and physiological decrements caused by sludges, and readjustments to these, summate over periods of years? How rapidly can they add up over short periods? How do these factors cumulate along with other nonsludge factors in the various aging processes?

The observations, experiments, and deductions outlined above are evidence that the sludges provide a common, easily understandable set of factors whereby many diseases can and do damage the bodies of animals and man. One great hope provided by these studies is that, as we learn how to keep blood normally unagglutinated and fluid, vessel walls intact, normal red cells from being destroyed, and adequate blood volume present, many effects of other pathologic mechanisms will stand out clearly, unobscured by the sludge mechanisms. Each will, of course, then receive the undivided attention it merits. The sludges are now ready for study by all the intensive investigative methods our age affords.

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NEWS *and Notes*

Robert T. Hatt, director, Cranbrook Institute of Science, will conduct an expedition to Yucatan this month to obtain land vertebrates. The work will be under the auspices of the Institute and the Viking Fund.

Hans Albert Einstein, engineer, U. S. Department of Agriculture Soil Conservation Service, has been appointed acting associate professor of engineering, University of California.

Harold F. Blum, physiologist, National Cancer Institute, has moved from the Marine Biological Laboratory, Woods Hole, Massachusetts, to the Department of Biology, Princeton University, where he has been appointed visiting lecturer.

William Walter Greulich, professor of anatomy, Stanford University School of Medicine, and director, Brush Foundation, has returned to the United States with Mrs. Greulich after spending three months in Guam and Japan. In Guam, Prof. and Mrs. Greulich completed a survey of the physical and nutritional status of Guamanian school children in collaboration with the Public Health Section, Naval Government, and as part of the Coordinated Investigation of Micronesian Anthropology, being conducted under the auspices of the Navy Department and the Pacific Science Board of the National Research Council. In Japan, they made a preliminary roentgenological and anthropometric study of children in Hiroshima and Nagasaki who were exposed to the irradiation from the atomic bombs. Similar observations were made also in Kure and Sasebo, whose populations resemble those of Hiroshima and Nagasaki, but which escaped bombing. This study was made for the Committee on Atomic Casualties, National Research Council, and conducted under the auspices of the Japanese National Institute of Health and with the cooperation of the Section of Public Health and Welfare of SCAP. The object of the study was to obtain information on which plans for a long-term investigation of the growth and development of the Japanese children in those areas can be based.

Otto Halpern, recently visiting professor of theoretical physics, Columbia University, has been appointed professor of theoretical physics, University of Southern California, where, in addition to graduate teaching, he will continue his theoretical research on neutron scattering and problems of interaction between matter and radiation.

Othmar F. Goriup, chief, Allotment and Requirement Branch, Medical Personnel Division, Office of the Air Surgeon, has been appointed the first chief of the recently created Medical Service Corps, Army Medical Department, with the rank of full Colonel.

Eliot C. Williams, Jr., who for the past 7 years has been associated with the Chicago Academy of Sciences, where he served as assistant director, has been appointed assistant professor of biology, Roosevelt College of Chicago.

Barry Commoner has been appointed associate professor of plant physiology, Henry Shaw School of Botany, Washington University, St. Louis.

Frances Carlin, formerly assistant professor of foods and nutrition, Hood College, Frederick, Maryland, has been appointed associate professor of foods and nutrition, Iowa State College.

E. M. Cralley, formerly associate professor of plant pathology, University of Arkansas, will return to the University in February 1948 as full professor. Dr. Cralley is now in Korea, where he is in charge of rice disease research for the military government.

George Neville Jones, associate professor, Department of Botany, University of Illinois, has been named associate editor of the *American Midland Naturalist* to represent the field of plant taxonomy.

Irvin E. Liener, First Lieutenant, Quartermaster Corps, U. S. Army, has been appointed to the staff of the Rations Planning Office, Quartermaster Food and Container Institute for the Armed Forces, Chicago.

Edward B. Ellis, formerly of Memorial Hospital, New York, has been appointed to the staff of the Technicon Company, 215 East 149th Street, New York.

Gerald G. Gross, former science writer on the *Washington Post*, is now editor of *Washington Report on the Medical Sciences*, a weekly newsletter with excellent coverage of happenings of a medical nature in and around Washington. Published every Saturday, it is timed for arrival at any point in the country on the following Monday. The subscription rate is $50 per year. A trial four-month subscription may be taken at the special rate of $15. Offices of *Washington Report* are at 1713 K Street, N. W., Washington 6, D. C.

Shields Warren, a pathologist at New England Deaconess Hospital and assistant professor of pathology, Harvard University, has been named interim director of AEC's new Division of Biology and Medicine (*Science*, Septem-

26, p. 285). Dr. Warren will be on half-time basis pending appointment of permanent director.

Horace M. Miner, associate professor, Department of Sociology, University of Michigan, has also been appointed associate professor in the Department of Anthropology.

Peter A. Bungart, preparator and collector of fossil fish, Department of Paleontology, Cleveland Museum of Natural History, has retired after 24 years with the Museum. **Harry C. Oberholser,** curator of Ornithology at the Museum, has also retired.

P. A. Wells, general secretary, Society of Dyers and Colourists, Bradford, England, has been appointed director of education, International Wool Secretariat, London.

Grover L. Bridger, chief, Process Development Division, Tennessee Valley Authority, has been appointed professor and head, Department of Chemical Engineering, Iowa State College, effective November 14. **O. R. Sweeney,** head of the Department since 1920, is retiring from administrative duties but will continue as professor of chemical engineering.

J. M. Schopf, formerly paleobotanist, U. S. Bureau of Mines, Central Experiment Station, Pittsburgh, has recently returned from the Union of South Africa, where he was engaged in petrographic study of coal under the auspices of the South African Council for Scientific and Industrial Research in cooperation with the South African Geological Survey and the Fuel Research Institute. Since returning, Dr. Schopf has been appointed to the U. S. Geological Survey, Section of Paleontology and Stratigraphy, with headquarters at 330 U. S. National Museum, Washington, D. C., where he will continue studies dealing with the constitution and microfossils of coal.

Max Lauffer, professor of physics, University of Pittsburgh, has been appointed chairman of the Executive Committee of the Department of Physics for the current academic year. **David Halliday** will serve as executive secretary for the Department.

Russell W. Cumley, medical editor, Abbott Research Laboratories, North Chicago, has been appointed to the recently created position of medical editor and director of publications of the M. D. Anderson Hospital for Cancer Research.

Joseph P. Fulton, University of Illinois, has been appointed assistant professor in the Department of Plant Pathology, University of Arkansas, where, in addition to teaching, he will conduct research on vegetable crop diseases.

Visitors to U. S.

Gregg M. Sinclair, president, University of Hawaii, recently visited the University of Texas Medical Branch, Galveston, to survey current educational and research methods in medicine.

H. A. Hyde, keeper of the Department of Botany, National Museum of Wales, Cardiff, summarized his studies on atmospheric pollen in the British Isles at the seminar of the Department of Botany, University of Minnesota, October 2. Mr. Hyde is visiting this country under the auspices of the Welsh National School of Medicine, Cardiff.

B. C. J. G. Knight, Department of Biochemistry, Wellcome Physiological Research Laboratories, Beckenham, Kent, England, will deliver the second Harvey Lecture of the current series at the New York Academy of Medicine on November 20. Dr. Knight will speak on "Bacterial Chemistry and Evolution."

Grants and Awards

The John and Mary R. Markle Foundation has embarked upon a new program to relieve the shortage of instructors in medical schools and to provide more trained investigators for medical research. Accordingly, the Foundation is offering post-fellowship grants to young scientists with the necessary training to hold regular faculty appointments and to conduct original research. Candidates, to be limited to young men and women with a particularly strong interest in research and teaching in any of the clinical or preclinical sciences or in the sciences basic to medicine, will be recommended for the grants by the medical schools, and the final choice of "Scholars in Medical Science" will be made on the basis of these recommendations and those of regional committees appointed by the Foundation. Grants of $25,000, payable to the cooperating medical school at the rate of $5,000 annually for a 5-year period toward the support of each successful candidate or his research, or both, will be available beginning with the academic year 1948–49. No fixed number of Scholars will be appointed in any one year, but it is expected that about 50 will receive appointments during the 5-year period. If the plan is successful, $1,250,000 will be appropriated to the schools by 1953. The program is the result of a survey of medical research and education made recently by the Foundation, which indicated that although various forms of financial aid are available to the student in the course of his scientific training and to the scientist once his name is made, there are few sources of help at the start of a career in academic medicine. Those interested may secure further details of the plan from the dean of the accredited medical school of their preference.

The American Pharmaceutical Association is now receiving nominations for the Iodine Educational Bureau Award ($1,000 and a diploma) which recognizes outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine. Any member of the Association may propose a nominee by submitting specific identification of the work to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight such copies must be submitted to the secretary of the Association, Robert P. Fischelis, 2215 Constitution Avenue, N.W., Washington 7, D. C., before January 1. The nominee must be a resident of the United States or Canada, must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine, including diagnostic use, and must have been actively engaged in, have completed, or have published a report upon the line of investigation for which the award is made, and he must not have been engaged in research under the sponsorship of the Iodine Educational Bureau, Inc., during the last two years.

George W. Vinal, chief of the Electrochemistry Section, National Bureau of Standards, has been awarded the Certificate of Commendation by the Bureau of Ships of the Navy Department for his outstanding service to the Navy during the war in development of new types of batteries and for his work as secretary of the Joint Army-Navy Battery Advisory Committee.

Edward R. Schwarz, head, Textile Division, Massachusetts Institute of Technology, was awarded the Olney Medal for outstanding achievement in the field of textile chemistry at the annual convention of the American Association of Textile Chemists and Colorists, held in Chicago October 23–25.

W. A. Pennington, chief chemist and metallurgist, Carrier Corporation, Syracuse, New York, was presented the Henry Marion Howe Medal for 1947 by the American Society for Metals at its annual banquet held October 23 in conjunction with the National Metal Congress and Exposition. The award is made annually to the author or authors of the technical paper of highest merit published in the annual *Transactions* of the Society. The subject of Dr. Pennington's paper was "A Mechanism of the Surface Decarburization of Steel."

Robert Jemison van de Graaff, Massachusetts Institute of Technology, has been awarded the 24th Duddell Medal of the Physical Society, London, in recognition of the invention and development of his high-voltage electrostatic generator. The Duddell Medal is awarded annually, without restriction as to nationality or to Fellowship in the Society, to persons who have contributed to advancement and knowledge by the invention or design of scientific instruments or by the discovery of materials used in their construction.

Frederick H. Verhoeff, professor emeritus of ophthalmic research, Harvard Medical School, and consulting chief of ophthalmology, Massachusetts Eye and Ear Infirmary, received the Leslie Dana Gold Medal presented by the American Academy of Ophthalmology and Otolaryngology, October 16, in recognition of outstanding achievements in the prevention of blindness and the conservation of vision.

Fellowships

The Lalor Foundation Fellowship Program at the Marine Biological Laboratory, inaugurated in 1947, will continue in 1948. Postdoctoral summer fellowships in the fields of biophysics, biochemistry, and physiological chemistry will be available. The fellowships are designed primarily to aid promising young investigators who can make maximum use of the facilities and opportunities provided at the Marine Biological Laboratory. In addition to laboratory facilities, the grants are intended to cover approximately the living expenses of the investigators at Woods Hole and necessary traveling expenses to and from Woods Hole. The committee administering the fellowships consists of Eric Ball, Kenneth Cole, Charles Packard, A. K. Parpart, and C. W. Metz (chairman). Inquiries and applications should be addressed to the director of the Marine Biological Laboratory, Woods Hole, Massachusetts, and applications should be received by December 31, 1947.

Radcliffe College has announced the availability of the Helen Putnam Fellowship for Advanced Research, which carries a stipend of $2,000 for 11 months beginning October 1, 1948, with a possibility of renewal for a similar period. Each Fellow will be assigned room and board at cost in one of the Radcliffe graduate houses and will be expected to be in residence during the tenure of the fellowship; she will also be provided with all normal laboratory facilities. There are no age restrictions for this fellowship, but in general the Committee on Award will limit its choice to mature women who hold their doctorates or equivalent qualifications. Appointments will be limited to those candidates who can submit a plan of research that is already under way in the fields of genetics or mental health, broadly defined. Application forms, which must be submitted before April 1, 1948, may be obtained from the Committee on the Helen Putnam Fellowship for Advanced Research, Radcliffe College, Cambridge 38, Massachusetts.

The Mellon Institute has announced the establishment there of a comprehensive multiple research fellowship on orthopedic appliances by the Sarah Mellon Scaife Foundation of Pittsburgh. Under the guidance of orthopedists and with the cooperation of both leading organizations in the field and manufacturers of orthopedic appliances, broad scientific investigation and development relating to such appliances will be conducted. Particular attention will be given to problems of mechanical design, improvements in materials of construction, and methods of fitting braces and similar orthopedic devices. **John L. Young,** research specialist in metallurgy and mechanical engineering, who will head the program as Senior Fellow, will be assisted by **Eugene F. Murphy,** staff engineer, Committee on Artificial Limbs, National Research Council, as Advisory Fellow and several research assistants. The medical advisory committee of the fellowship is under the chairmanship of Paul B. Steele, professor and head, Department of Orthopedic Surgery, School of Medicine, University of Pittsburgh, who will be assisted by John A. Heberling, associate professor of orthopedic surgery, and Carl C. Yount, assistant professor of orthopedic surgery, both of the University of Pittsburgh.

Industrial Laboratories

William W. Hartman, assistant superintendent, Synthetic Chemistry Department, Eastman Kodak Company, has been appointed superintendent of that Department.

George H. Schneller, formerly head of pharmaceutical research, Wm. S. Merrell Company, Cincinnati, has been appointed director of pharmaceutical application in the Pharmaceutical Department, Calco Chemical Division, American Cyanamid Company, Bound Brook, New Jersey.

Alton Bryant, who recently obtained his Bachelor's degree in bacteriology from the University of Kentucky, has joined the Bacteriology Laboratories of the Wm. S. Merrell Company, Cincinnati.

A course in X-Ray Diffraction and Spectrometry will be given by the North American Philips Company, Inc., November 17–21, at its New York offices, 100 East 42nd Street, to acquaint industrialists, technicians, and research specialists with fundamentals of X-ray diffraction and its applications. Lectures will be given daily, followed by discussion periods and laboratory work. Guest speakers will include: M. J. Buerger and John Norton, of Massachusetts Institute of Technology; Charles Barrett, University of Chicago; I. Fankuchen, Brooklyn Polytechnic Institute; Herbert Friedman, Naval Research Laboratory; and William Parrish, Philips Laboratories, Inc. Sessions will begin at 9:30 A.M. and end at 5:00 P.M. A fee of $50 is required of all taking the course except the Philips diffraction equipment owners.

The Nucleonics Project of General Electric Company, which consists of work on atomic energy for the U. S. Atomic Energy Commission, now has a

its administrator **R. S. Neblett,** who has been manager of G-E's Federal and Marine Divisions. The G-E Nucleonics Committee, which has administered the Company's activities in this field for the past year, has announced that responsibility for contractual administration and negotiations in connection with the Project is transferred to the Chemical Department. The Committee will retain responsibility for general policies of administration and operation of the Project. Operation of the $20,000,000 Knolls Atomic Power Laboratory, now under construction by the Company for the AEC, will remain under the G-E Research Laboratory, while the Chemical Department will retain responsibility for operation of the Hanford Engineer Works near Richland, Washington.

Colleges and Universities

The 29th course of Lane Medical Lectures, which were founded in 1896 by the late Levi Cooper Lane, will be given each evening at 8:15, November 11–13 and 17–18, in Lane Hall, Stanford University School of Medicine, Sacramento Street, near Webster, San Francisco, California. The lectures will be delivered by Wilder G. Penfield, professor of neurology and neurosurgery, McGill University, and director, Montreal Neurological Institute, on the subject, "Physiological Observations on the Cerebral Cortex of Man." The 5 individual lectures on this topic will include: "Introduction, With Observations on Hearing and Vision," "Sensorimotor Organization of the Cerebral Cortex," "Secondary Mechanisms of Movement, Sensation, and Speech," "Dreams, Hallucinations, and Memory," and "Cortex, Consciousness, and Cerebral Seizures." The medical profession, students, teachers, and research workers in medicine and allied sciences are cordially invited to attend.

The Academy of Natural Sciences, Philadelphia, has set up a cooperative arrangement which enables graduate students of Bryn Mawr College, Haverford College, LaSalle College, Swarthmore College, University of Pennsylvania, Temple University, and Rutgers University to study systematics as a part of their regular curriculum. **Ruth Patrick,** associate curator of Microscopy at the Academy, **Francis W. Pennell,** curator of Plants, and **H. Radclyffe Roberts,** managing director of the Academy, are in charge of the course. Emphasis will be on the principles of systematics rather than on the classification of any particular group. Cytological, serological, physiological, and statistical, as well as morphological methods will be considered. The course is given at the Academy every Saturday from 8:00 A.M. to 1:00 P.M.

Meetings

The Tennessee Academy of Science will hold its 57th annual meeting November 28–29 at the University of Tennessee, Knoxville. Paris B. Stockdale, Department of Geology and Geography, University of Tennessee, president of the Academy, will preside at the opening general session Friday morning. Sectional meetings will convene Friday afternoon under the leadership of the following chairmen: Botany, D. M. Brown, East Tennessee State College; Chemistry, Raymond Seymour, University of Chattanooga; Geology and Geography, Harry J. Klepser, University of Tennessee; Mathematics, T. C. Carson, East Tennessee State College; Zoology, C. L. Baker, Southwestern University; and the Junior Academy, Frances R. Bottom, sponsor, George Peabody College. The annual dinner will be held Friday evening, and the business session with election of officers, Saturday morning. Members wishing to submit papers at the general sessions should send titles to the program chairman, Arthur C. Cole, Department of Zoology, University of Tennessee. Those wishing to present papers at the sectional meetings should submit titles to the sectional chairmen.

The American Physiological Society will hold its regional meeting as an open symposium on "Military Physiology" at the Army Medical Department Research and Graduate School, Army Medical Center, Washington, D. C., December 4–6. Members of the Society, their associates, and students are invited to attend. At the first session, beginning at 10:30 A.M. in the Sternberg Auditorium, Army Medical Center, representatives of 9 Army, Air Force, and Navy laboratories will present the high lights of their programs in physiological research. The second day will be devoted to the presentation of research on respiratory physiology carried out with military support in 10 universities. At the last sessions, papers will be presented on physiological researches conducted in neighboring government and civilian laboratories.

The 15th annual meeting of the Association Canadienne-Française pour l'Avancement des Sciences was held October 11–13 in Montreal, Canada. Papers were presented in the Physics, Chemistry, Geology, General Biology, Entomology, Botany, Agronomy, Biogeography, and the Moral Sciences Sections. A report of two symposia on the scientific training in French Canada and on the situation of French-Canadians in scientific positions will be published shortly. The BAAS was officially represented by its president, Sir Henry Dale, and the AAAS by J. W. Bridges, professor of experimental psychology, Sir George Williams College, Montreal. Joseph Risi, professor of chemistry, Laval University, Quebec, was elected president; Léon Lortie, professor of history of science, University of Montreal, vice-president; and Lionel Lemay, professor of chemistry, University of Montreal, general secretary. The main office of the ACFAS is located at the University of Montreal, 2900 Mount-Royal Boulevard, Montreal 6, Canada.

The Hungarian Research Institute for Genetics and Plant Breeding, Budapest, has sent a request to the Committee on Aid to Geneticists Abroad, Genetics Society of America, for reprints in genetics, and particularly in plant genetics. Packages of reprints weighing not more than 4⅔ pounds (2 kilos) may be sent to Dr. Thomas P. Bogyó, Research Institute for Genetics and Plant Breeding, Hungarian Bureau of Agricultural Science, II Herman Otto út 15, Budapest, Hungary.

The American Chemical Society has chartered its second local unit outside the United States, this one being in Puerto Rico. Officers of the new section are: chairman, Fritz Fromm, professor of chemistry, College of the Sacred Heart; chairman-elect, Fernando Badrena, production engineer, Puerto Rico Phosphate and Acid Works, Inc.; and secretary-treasurer, Leonardo Igaravidez, Agricultural Experiment Station, University of Puerto Rico. Víctor Rodríguez Benítez, chairman, Department of Chemistry, Agricultural Experiment Station, will represent the section on the Society's national council.

COMMENTS
by Readers

Because I believe the President, the Congress, and most scientists are in agreement on the essentials of national science legislation and administration, I venture to suggest that differences relating to the setup of the proposed Foundation and the details of its administration and operation should be settled promptly so that we may get on with the business of producing scientists and fostering research on a civilian basis. The points on which we seem to be in agreement are the following:

Freedom of research and education. It is accepted by most scientists that it would be a mistake to establish a foundation which would attempt or be tempted to select and supervise the particular projects on which scientists shall work. It is agreed generally that, given reasonable provision for accounting for the use of public funds, the institutions and the individuals within institutions selected to carry on research should be granted freedom of choice and action. This is not to say there should be no "request" research, but the emphasis should be on "free" research. The same applies to individuals granted scholarships or fellowships: once it is decided by competent authorities that they have capacity for scientific education or advanced research such as to warrant support, scholars and fellows should be free to pursue their interests in institutions and in directions of their own selection.

Civilian administration. The Army and Navy have been in a position since the war to command support for research not immediately related to military requirements, and have had the guidance of scientists tried and qualified by wartime and other experience. This effort will go forward under the new military setup with Dr. Bush as chairman of the coordinating board for the three Services. Despite the temptation, in view of the controversy over the National Science Bill, to acquiesce in continuation of an arrangement of proved efficiency, I believe none of us is yet willing to default in favor of permanent military administration. Granting the need for military research on weapons and materials, and the necessity for cooperation between military and civilian authorities, it seems to be conceded that civilian rather than military administration of most of the basic research is desirable.

Emphasis on fundamental research in universities and colleges. This proposition does not require argument in *Science.* The point that should be mentioned is that "universities and colleges" does not mean merely the big universities and colleges, nor does it mean institutions located in certain sections of the East, Middle West, and West. I have never met a scientist serving as head of a university or institution, or engaged in research or teaching, who did not say that the hothouse methods employed during the war should be greatly modified, if not abandoned, in the interest of making it possible, as soon as possible, for any college or institution, and for any individual anywhere in the country, to qualify for support. Most of the scientists I know would gladly risk a waste of funds rather than sacrifice the opportunity to build up scientific research and education throughout the country. They know that research capacity and scientific aptitude are not localized phenomena. What they oppose is provision for mandatory, arbitrary distribution of funds without reasonable reference to scientific or educational standards.

Emphasis on training personnel. This point is mentioned only to show it has not been overlooked. For several years the principal object must be to produce scientists and, particularly, teachers of science.

Utilization of both laymen and scientists in the program. There is some misunderstanding about this. It has been assumed by many that the National Science Board, under S. 526 or S. 1850 (the rival legislation), would consist only of scientists, and that the persons appointed would be heads of institutions or departments or other "big-name" men. There is no basis for these assumptions. The bills that have been seriously considered provide for the appointment of qualified laymen as well as scientists on the basis, without reference to politics, of capacity to serve and promote the interests of the Foundation. That qualified laymen should be attracted to this service is not denied except perhaps by those few who believe that laymen having private interests are incapable of giving disinterested and effective service to a public agency.

Agreeing that it is quality of administration we are looking for, it is generally accepted that we should not insist on filling the board only with those who are able and willing to become full-time officials. Room should be left, and is left in the bills under consideration, for the appointment of present employees of the Government and for recruiting laymen as well as scientists outside the Government.

We cannot dodge the fact that there have been conflicts among us over certain features of the legislation. It is on these points that reasonable concessions should be made:

Appointment and responsibility of the director. It may be that in this respect the bill rejected by the President violates "basic principles which make for responsible government"—but I doubt it. The highly successful National Advisory Committee for Aeronautics is an agency similar in essential respects to the Foundation proposed in the rejected bill. (In the case of NACA there would be more reason for application of the "in-line" principle: NACA is an operating agency, whereas the proposed Science Foundation is precluded from conducting laboratories or pilot plants.) Likewise in the states there are departures from the principle. In New York, for example, the Commissioner of Education is appointed by, and is responsible to, the Board of Regents; I need but mention two recent incumbents—President Stoddard, of the University of Illinois, and his successor, Commissioner Spaulding—to demonstrate that successful administration is not dependent on a theory of organization.

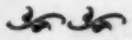
The object is to establish a Foundation which will be vital and imaginative and liberal, not merely efficient in a bureaucratic sense. Nevertheless, those of us who were satisfied with S. 526 should be the first, perhaps, to offer a concession in view of the strong and authoritative view the other way. None of us wants to dilute

or disparage the powers and responsibilities of the President; what is really desired is that the scientists represented on the board shall have a voice in the choice of the principal executive officer of the Foundation. An amendment which would give the President the power to appoint the director after receiving nominations from the board, and which would give him the power to remove the director, would substantially meet the objection to this feature of S. 526.

Status of the board. It has been said that the board described in S. 526 would consist "essentially of private citizens" who would meet only occasionally, and it is suggested that their service would be casual and perfunctory. There is no basis for this view. All of our experience demonstrates that if strong appointments are made, the members will be conscientious to the point of sacrifice—as much so, at least, as the members of any full-time commission now serving in Washington. One of the principal reasons for specifying a part-time board was to obviate the drag which, after the formative period, affects full-time commissions; to save as much as possible of the amateur spirit in the direction of the Foundation; and to attract men and women, scientists and laymen, who might be unable to devote full time to Foundation service. It is hoped the critics of S. 526 will make a concession on this point.

It has been too little stressed, I think, that in making this provision for Federal grants to institutions and individuals we shall be better satisfied if ultimate responsibility is placed on the shoulders of a selected group of our fellow citizens rather than in the hands of a full-time official. Without reflecting unfavorably on the present administration of taxpayers' money for scientific research and education, it should be borne in mind that we are proposing a vast extension of Federal assistance which, I submit, should be subject to direction and check beyond that required for the ordinary business of Government. Related to this is the belief held by many that, to minimize if not avoid political interference and criticism, the President and the Director of the Foundation should be protected against pressure for grants; an authoritative board appointed by the President should be responsible for policies and grants.

Other details. Though the President is critical of other features of S. 526, the Congress and the scientists do not seem to be involved in any serious disagreement. The provision for the interdepartmental committee should be amended to place the direction of its activities directly under the President's authority. The provisions for special commissions (except perhaps the provision for a commission on cancer research, which might serve a useful purpose in establishing a clearing and coordinating agency) seem unnecessary and should be eliminated. (BETHUEL M. WEBSTER, *15 Broad Street, New York City.*)



Work done in compression, pdv = dW, say, has as its counterpart a change in potential, vdp = dW_0; similarly for a change in thermal energy, dQ = TdS, and its counterpart, SdT = dQ_0. It is proposed to regard this dQ_0 as potential thermal energy analogous to work potential vdp and so clarify and simplify the thermodynamics of deformation.

In a previous paper (*Science*, October 4, 1946, p. 317) it was shown that the Second Law in a very simple and useful form may be directly derived from the Gibbs thermodynamic potential, U − TS +·pv, for any body in which that potential is uniform. When energy dU (either thermal or mechanical) is added or removed,

$$(1) \qquad dU - TdS + pdv = 0$$

by the First Law; hence

$$(2) \qquad SdT = vdp,$$

the Second Law.

In other words, as the internal energy of a body is changed, whether by heat or by mechanical work, the thermal and mechanical potential energies change always by equal amounts. The "free" energies, TdS and pdv, are not equal in general, but SdT = vdp ($dQ_0 = dW_0$) for every reversible process.

Free and potential energies are related through the physical properties of a body. For example, pdv/vdp = $p\beta$, where β is the compressibility defined by $dv = v\beta dp$. Similarly, TdS/SdT = αT, where α is the thermal coefficient of expansion given by $dv = v\alpha dT$.

$$(3) \qquad \frac{pdv}{vdp} \equiv \frac{d \log v}{d \log p} = \frac{dW}{dW_0} = \frac{dW}{dQ_0} = p\beta,$$

$$(4) \qquad \frac{TdS}{SdT} \equiv \frac{d \log S}{d \log T} = \frac{dQ}{dQ_0} = \frac{dQ}{dW_0} = T\alpha,$$

$$(5) \qquad \frac{Tdp}{pdT} = \frac{TdS}{SdT} = \frac{dQ}{dW} = \frac{T\alpha}{p\beta}.$$

These sets of fundamental relations permit many kinds of transformations between variables, but adiabatic coefficients must not be confused with isothermal.

In the writer's proposed general law of deformation (see *J. Franklin Inst.*, May 1921 and December 1946),

$$(6) \qquad \frac{dy}{y} = n\frac{dt}{t} + m\frac{dp}{p} + r\frac{dT}{T},$$

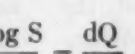
the parameters n, m, r are ratios of fractional increments similar to those in (3), (4), and (5) above. If y in (6) is volume, then m = $p\beta$ and r = $T\alpha$. Hence, by (6), m is simply the ratio of the increments of free and potential mechanical energy, dW/dW_0, and r is dQ/dQ_0. The creep factor involved in n of (6) is time t times a constant having the dimension reciprocal time.

The relations discussed above are, of course, exact only in the differential form given. Some of them hold in integral form over a surprisingly wide range, but in such cases the physical processes involved must remain constant. And in the differential form (6) there is no difficulty with fractional dimensions, since the parameters are simply dimensionless ratios of fractional increments, each of which is dimensionless.

Since the increments of thermal and work potential are always equal, whether due to added heat or work, it follows that the total potentials remain equal over very wide ranges.

In deriving his radiation formula, Planck found the probability of P packets of radiation, each of energy $h\nu$, being associated with N resonating particles having an average energy E_0, introducing the assumption $Ph\nu = N E_0$, or radiation density equals mechanical energy density. Fitting this assumption to the Second Law (2) involves some interesting physical relations.

For gases, $T\alpha$ and $p\beta$ are unity; hence, in a gas all four forms of energy are present in equal amounts. For most solids and liquids these products are very small, and correspondingly large potentials are to be dealt with. (P. G. NUTTING, *3216 Oliver Street, N.W., Washington, D. C.*)



TECHNICAL PAPERS

The Remote Sustained Threshold Therapeutic Action of Streptomycin in Tuberculosis

H. J. CORPER and MAURICE L. COHN

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The use of streptomycin as a therapeutic agent in tuberculosis has posed many problems. It is believed such problems cannot be solved by human therapeutic use but, of necessity, must be founded basically in the intimate mechanisms of its actions in tuberculosis. Therefore, exacting and carefully planned animal experimentation will be required for elucidation. Although suspected of acting in the animal economy as it does in the test tube, there are many discrepancies between so simple an explanation and observed facts.

In previous communications (*1*, *2*) it was shown that streptomycin is not capable of destroying either virulent or avirulent human tubercle bacilli in the body and that maximum tolerated doses in guinea pigs (or other laboratory animals) cannot completely retard the development of the tubercle bacilli or tuberculosis following intravenous infection. However, it does exert a definite and fairly consistent partial retarding effect on the development of tuberculosis in animals in appropriate doses, and therefore must be considered an adjuvant treatment for this disease at present under certain limitations prescribed by necessary sanatorium and hospital regimes or supervision.

Streptomycin cannot be considered an inert or nontoxic therapeutic agent in itself, and therein lies the question of the appropriate, the maximum, and the most efficient dose to attain the maximum therapeutic benefit, acknowledging that it is an adjuvant agent against tuberculosis in man. On the toxicological and pathological side there is every reason to believe that those early enthusiasts who saw little detrimental effect from its use for short periods of time considered only spectacular effects in man as significant. It was noted that "early side reactions have not been alarming, and no late toxic effects have so far been observed" (*7*); or, "In this limited experience, tests of renal and hepatic function together with blood studies before and after the parenteral administration of streptomycin revealed no evidence of serious toxicity. Reactions, consisting of fever, arthralgias, and skin rashes as well as histamin-like effects, are believed to be due to impurities retained in the preparations of streptomycin employed in these studies" (*4*). A better interpretation of the toxicity, in 1946, seemed to be that "streptomycin as well as its impurities are pharmacodynamically very active compounds. A more detailed knowledge of their toxicologic properties is a matter of great importance" (*5*). Reporting more recently from studies with streptomycin administration in tuberculosis in man, the conclusion is drawn that "as streptomycin can produce potentially serious toxic reactions it is inadvisable to use the drug in the treatment of generally benign infections, such as recently acquired pulmonary tuberculosis of minimal extent (*3*). A more strictly pathological study on normal animals (monkeys, dogs, rats, mice, chickens, and guinea pigs) indicated a toxicity of low order, but large doses produced focal necroses in the lungs in dogs, fatty metamorphosis in the kidneys of monkeys and dogs, and neurotoxic effects in dogs (*6*).

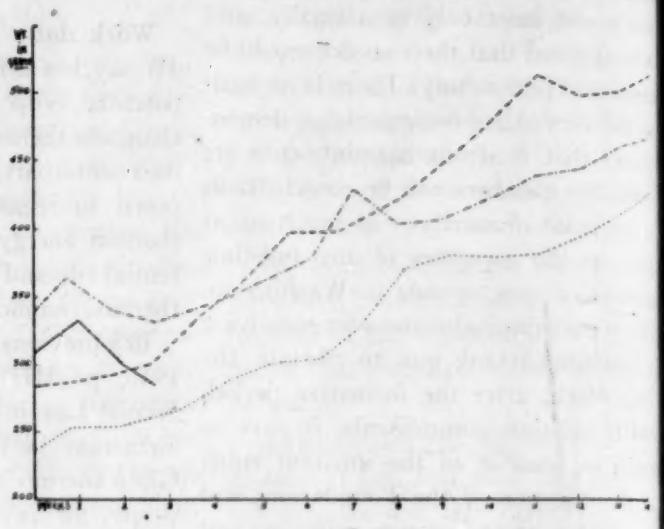

FIG. 1. Average weight chart of streptomycin-treated animals: —— infection controls; ---------- 4 daily doses; — — — 1 daily injection; — — — 5-day interval injection.

The following report on the effect of streptomycin in tuberculosis is not intended to be final or complete and is presented with the full recognition of a definite effect of streptomycin as a partial retardant to tuberculosis. For this presentation it is limited to the guinea pig, the animal in which this retardant effect of streptomycin was first demonstrated. The information disclosed may be valuable for the proper therapeutic use of streptomycin in man as well as extending the appropriate use of this drug, which is still limited in production and economically beyond the reach of some institutions and tuberculous individuals. It must be recognized at the outset that these results are those of a set, controlled experiment in which the bacilli were introduced into young, normal animals. Such effects of streptomycin on tuberculosis as are noted here might occur in the naturally infected and relatively immune tuberculous man far less definitely and consistently, although the significance of the dosage of streptomycin would in all probability be the same and could be applied to man without exaggeration. If we now use the two most conspicuous effects of streptomycin in tuberculosis (the prolongation of life and the retardation of the disease in the guinea pig) as a criterion, it will be possible to approximate the relative value and efficiency of different modes of treatment, comparatively speaking, with streptomycin in tuberculosis. Without elaborating upon details, the following findings with streptomycin treatment were noted, using for this study only the best, purest, and highest unit titer streptomycin obtainable at present. All the streptomycin used rated at about 1,000,000 units/gram.

In all the infections in these experiments, a highly virulent strain of human tubercle bacilli (#4008) was used, and a standard amount (1 mg.) of fine suspension was injected intravenously into the ear vein. All treatments with streptomycin were given by subcutaneous injection.

In a series of preliminary experiments in which the persistence of streptomycin effect was studied, using a sufficiently large number of controls (6) and an equal number of streptomycin-treated animals (given 25,000 units of streptomycin daily for 82–91 days and infecting intravenously one day after the last treatment), the average duration of life of the controls was 22 days compared with 29 days for the pretreated animals. When the pretreatment with a similar amount of streptomycin daily continued for only two weeks, the average duration of life of the controls was 21 days; and for animals pretreated with streptomycin, it was 22 days, which is about the same, although the spleen size was preceptibly less in the treated animals. In single injections, 50,000 units of streptomycin were not lethal, while 100,000 units were frequently fatal in our tests on normal animals.

In order to compare the results of the injection of 4 doses of streptomycin daily for a total of 25,000 units with a single injection of 25,000 units daily and 25,000 units given at 5-day intervals in a single injection, several sets of duplicate experiments were performed. Four to 6 or more guinea pigs were used in each test as well as infection control animals given only the intravenous injection of 1 mg. of virulent human tubercle bacilli in fine suspension without treatment with streptomycin. While the average duration of life of the control infected guinea pigs varied within the narrow limits of 19–21 days, that of the animals given 4 daily injections to a total of 25,000 units daily was from about 100 to about 150 days. Those given the 25,000 units at 5-day intervals, starting 6 days prior to infection and continuing throughout infection, were also alive beyond 100 days in most instances.

In these experiments it was noted that individual animals would die of a generalized tuberculosis in spite of intensive treatment with streptomycin and regardless of whether the injections were given daily or at 5-day intervals.

The appended 14-week weight graph (Fig. 1) illustrates the effects of 4 daily injections (for a total of 25,000 units) of streptomycin, one single daily injection throughout the experiment, and single injections of streptomycin at 5-day intervals compared with infection controls. In the series in which the streptomycin was given only as a single injection of 25,000 units at 10-day intervals, the treated animals outlived the controls, so that the average of the controls was 20 days and that of the treated 70 days.

The foregoing experiments would appear to indicate that streptomycin does not act in tuberculosis as a simple chemotherapeutic retardant as it does in the test tube (since simple *in vitro* acting and *in vivo* distributed chemicals fail to affect tuberculosis), but that there is a threshold of remote sustained action. When initiated minimally, the effect persists for some time; above the maximum threshold effect, it is needless to continue forcing treatment, since the benefit derived does not exceed that of the established maximum. This information should extend the present use of streptomycin as an adjunct to the treatment of human tuberculosis and make streptomycin available for human treatment where excessive administration previously was economically prohibitive to certain cases.

It would appear that the amount and frequency of administration of streptomycin in tuberculosis can be reduced without appreciable loss of effectiveness. It is felt that the intimate mechanism of streptomycin action in tuberculosis still remains to be disclosed satisfactorily.

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A Possible Role of Food Purification in the Etiology of Dental Caries

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A study (*3*) has been made of factors influencing the caries susceptibility of rodents (36 mice, 53 Syrian hamsters, and 153 Long Evans strain rats). It has been possible to produce dental caries similar to that in man by feeding a finely powdered, purified ration (*1*) containing 67 per cent purified carbohydrates, primarily sucrose, and complete in known nutritional essentials. Caries did not develop in the mice and rats and was limited in the hamsters unless the experimental feeding on the purified diet was commenced during tooth development.

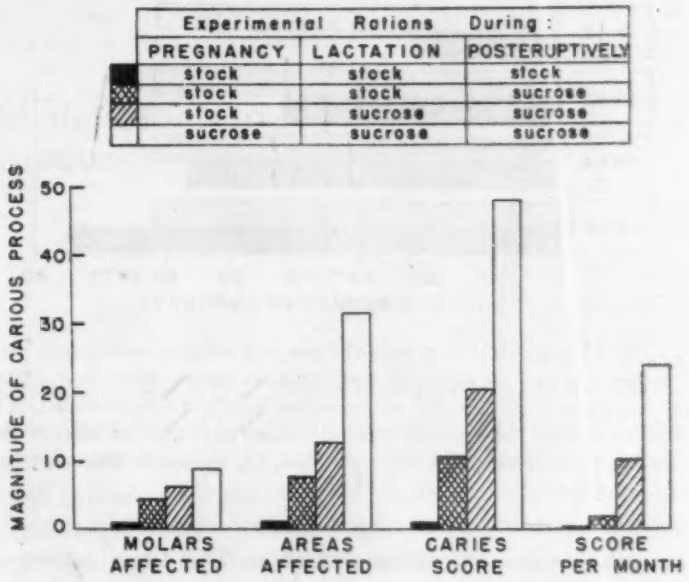

Fig. 1. The caries incidence of groups of hamsters whose experimental feeding on the purified ration was commenced *before*, *during*, and *after* tooth developments, respectively; their molars decayed in a ratio of 20:10:2. ("Sucrose" diet refers to the purified ration, containing 67 per cent sucrose. "Stock" diet refers to the Purina laboratory chow.)

The caries susceptibility appears to be greatly influenced by a mechanism (possibly an unrecognized nutritional factor) operating before tooth eruption from conception to maturation of the offspring and their individual teeth (see Fig. 1). In the light of these results a reinterpretation has been made of earlier observations in animals and man.

A review of previous failures to produce caries in various animals (*3*), including the primate, fed purified high sucrose diets suggests that the experimental feeding started out with

too old animals, did not extend to second generations, or covered too short periods within one to overcome caries-resisting properties of the species, strain, or teeth in question.

An over-all analysis has been made of the trend of dental caries in about 800,000 children (*2*) surveyed by various workers in 11 European countries during the last 50 years. Following drastic reductions in consumption of refined carbohydrates at the beginning of the two World Wars, a marked caries reduction can be demonstrated. But the time relationship between the decrease in sugar consumption and the reduction in caries cannot be explained on the basis of a rapid mechanism in the oral environment. There is a delay of several years in the initial effect and a lag of many years in the terminal "effect

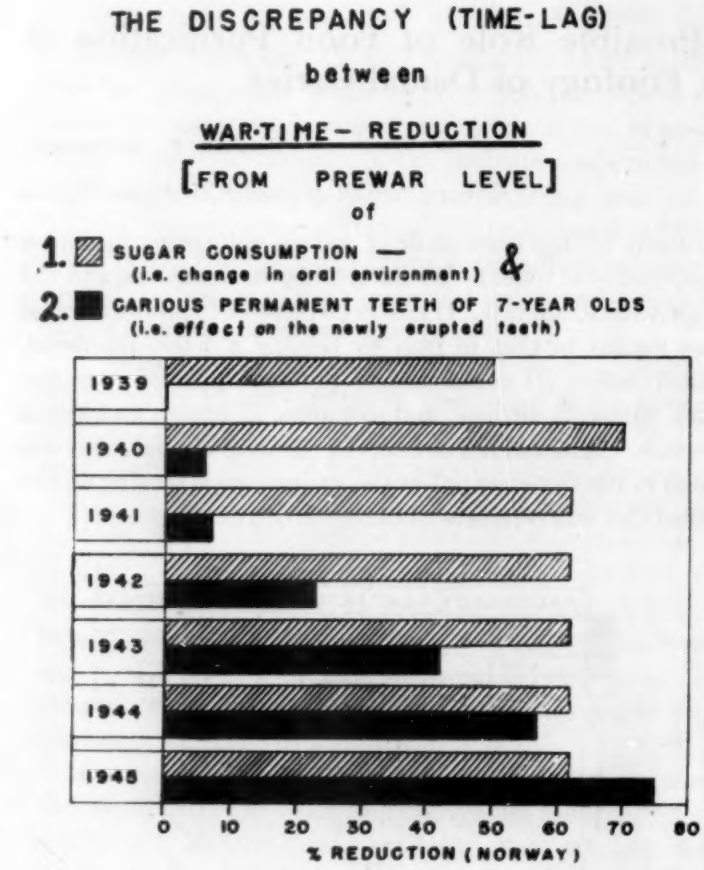

FIG. 2. A comparison of groups of 7-year-old children during each year of the war. Their permanent teeth *after eruption* were exposed to a similar sugar concentration in the oral environment. Significant caries reduction is seen in the teeth of those groups (1943/45) whose teeth had the longest "exposure" to the wartime diet *before eruption, i.e.* during development and final maturation.

of the wars" upon the caries reduction. This time factor can best be explained by an accumulative favorable effect before the teeth erupted into the oral environment (Fig. 2).

The mechanism of this beneficial influence upon the teeth developing during the war is not known. It cannot be attributed to any obvious, over-all uniform increase in the consumption of any previously demonstrated caries-inhibiting food or food factor, but may be related to the fact that the bulk of the caloric intake during the wars was in the form of natural foods. The large caloric loss resulting from the sugar and fat rationing primarily seems to have been compensated for by an increased consumption of nonpurified carbohydrates, potatoes, cabbage, and wartime bread (containing 80–95 per cent of the grain).

In view of these experimental and clinical observations, it is possible that the long-suspected relationship between dental caries and the excessive consumption of refined carbohydrates may be in the nature of an unrecognized indirect influence upon the quality of the offspring and its developing teeth, and that the effect may be accumulated through generations.

The hypothesis of an accumulative depletion of a nutritional factor or combination of factors favorable to the developing teeth is being tested.

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Effect of Tetraethylammonium Chloride in Experimental Gastric Ulceration in the Rat

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Recently a method has been described for the uniform production of gastric ulceration in the rat (*4*). This method, or a modification of it, has been used to test the effectiveness of antiulcer factors (*5*). Harkins has reported (*2*) the complete prevention of gastric ulceration in rats by transabdominal vagotomy, using this method.

It has been shown (*1*) that the tetraethylammonium ion blocks the transmission of impulses at autonomic ganglia. In view of the reported effectiveness of vagotomy in preventing gastric ulceration in the rat and the widespread use of vagotomy in the clinical treatment of peptic ulcers, it was thought that the tetraethylammonium ion might prove useful in the therapy of this condition. Therefore, the effectiveness of this compound in preventing gastric ulceration in the rat has been studied.

Eighteen female rats weighing 80–100 grams were fasted for 72 hours. The rats were kept separated in cages with a wide wire-mesh bottom and were given water throughout the fasting and experimental period. They were anesthetized with ether. A short midline incision extending downward from the xiphoid process was made and the pylorus carefully exposed and ligated. The incision was closed with sutures, and the wound was covered with a thin coating of flexible collodion.

Alternate rats were injected intramuscularly with 1 mg. of tetraethylammonium chloride (Etamon chloride,[2] 1 ml. of saline solution) just prior to the pyloric ligation and at hourly intervals thereafter until death or until the animal was sacrificed.

The results are summarized in Table 1. Rat #1 died as a result of anesthetization, and rat #9, a control, has been omitted from the series because it was found dead 2.5 hours after pyloric ligation and showed no lesions at autopsy. It

[1] The authors gratefully acknowledge the assistance of Woodrow Batten and J. I. Bumgarner in some of the experimental work.

[2] The Etamon chloride was generously supplied by E. C. Vonder Heide, of Parke, Davis & Company, Detroit, Michigan.

will be seen that all 7 of the controls died in less than 8 hours, with an average survival time of 6.7 hours. At autopsy, 6 of the 7 control rats showed perforation of the stomach. Of the 9 rats receiving Etamon chloride only 4 died; the shortest survival time was 7.1 hours, and only one of these 4 showed perforation. The remaining 5 experimental rats were sacrificed no sooner than 9.7 hours postoperatively, so that the shortest postpyloric ligation time was 1 hour longer than the longest control survival.

The stomachs were examined microscopically for ulceration. The results of this examination have been tabulated on the basis of an arbitrary division into two groups: (a) stomachs having ulcers in which one axis was at least 3 mm. long, and (b) stomachs having ulcers in which the longest axis was less than 3 mm. It will be seen that 6 of the 7 control rats were in

TABLE 1

Rat No.	Weight (gram)	TEAC* (mg.)	Survival (hr.)	Perforation	No. of ulcers: 3 mm. or greater — Rumen	Body	Antrum	Less than 3 mm. — Rumen	Body	Antrum
Control series										
3	90	0	4.8	+	1	0	0	5	0	0
5	95	0	4.6	+	5	0	0	0	1	0
7	90	0	6.8	0	1	0	0	1	0	0
11	82	0	8.7	+	0	0	0	11	0	0
13	90	0	6.7	+	1	0	0	1	0	0
15	100	0	7.4	+	3	0	0	17	0	1
17	100	0	7.8	+	2	0	0	9	0	0
Total			46.8		13			44	1	1
Average			6.7		1.9			6.3		
Experimental series										
2	95	13	12.8†	0	1	0	0	0	0	0
4	90	10	9.8	0	0	0	0	0	0	0
6	90	11	9.9	+	1	0	0	9	0	0
8	95	12	11.5†	0	0	0	0	8	0	0
10	90	11	11.2†	0	1	0	0	1	0	0
12	80	8	7.1	0	0	0	0	0	0	0
14	100	10	10.4†	0	0	0	0	0	0	0
16	88	8	7.8	0	1	0	0	0	0	0
18	95	10	9.7†	0	1	0	0	4	0	0
Total					5			22		
Average					0.6			2.4		

* Tetraethylammonium chloride.
† Rats were sacrificed after this survival time.

the group having large ulcers in the rumen, while this was true of only 5 of 9 experimental rats. Furthermore, 3 of the 6 control rats had more than one large ulcer. Six of the 7 control rats were in the group having small ulcers in the rumen, and in 4 of these the ulcers were multiple. Four of the 9 experimental rats had small ulcers in the rumen, and in 3 of these the ulcers were multiple. One control rat each had a small ulcer in the body and in the antrum of the stomach. Three of the experimental rats showed no gastric ulceration, while all of the control rats showed ulceration.

A comparison of the effect of Etamon chloride on gastric fluid volume and acidity was impossible due to the high incidence of perforation in the control series.

Although Etamon chloride did not completely prevent gastric ulceration in this series, on the basis of survival time, perforation, and incidence of ulceration it appears that it was definitely beneficial. When one considers the possibility that the difference between the two series might have been more striking had all remaining animals been sacrificed and examined after 7 hours (approximately the average survival time of the control rats), the probable clinical usefulness of Etamon chloride is enhanced.

Lyons, *et al.* (*3*) reported that in one hypertensive patient, who also had a duodenal ulcer, a single intramuscular injection of 1.2 gram of Etamon chloride resulted in the cessation of gastrointestinal motility, relief of the ulcer pain, and a decrease in the acidity and volume of the gastric juice. As the effects of the drug diminished, pain returned, at about 7 hours, and peristalsis was believed to be more rapid. We have studied the effect of Etamon chloride in two human patients who had active duodenal ulcers as well as in patients with other gastrointestinal disorders, with suggestive beneficial results. The clinical studies are being continued and will be reported elsewhere.

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A Method for Screening Antimalarial Compounds in the Mosquito Host

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Although methods of antimalarial drug screening have been improved and refined during the past several years, there has, nevertheless, been need for new screening technics, principally because it has not been possible to establish consistent or predictable relations between drug activity in experimental animal malarias and that in human malaria. For example, whereas sodium sulfadiazine and other sulfonamide derivatives have a kind of prophylactic effect against sporozoite-induced *Plasmodium gallinaceum* infections in chicks, these drugs are ineffective as prophylactic agents against human malarial infections. The present study was undertaken to devise a method of drug screening which would permit of more direct screening for certain drug qualities against a specific malarial parasite, or which would permit of screening of compounds for prophylactic effect against the human malarial parasite without the need for infecting undue numbers of human hosts.

In these experiments, laboratory strains of *Aëdes aegypti* were infected with a strain of *P. gallinaceum* designated as the 8A strain. Quinine hydrochloride, quinacrine hydrochloride,

plasmochin citrate, 7–chloro-4-(4-diethyl-amino-1-methyl butyl amino)-3-methyl quinoline bisulfate (SN6911), and sodium sulfadiazine were prepared in solutions calculated in terms of the salt. These drugs were added in various concentrations to a 4 per cent sugar nutrient solution used as the standard maintenance diet for infected mosquitoes in this laboratory. Mosquitoes fed readily on these drug mixtures, and, as in animal drug testing, mortality or survival was directly related to drug levels. It is evident that mosquitoes on these diets took up the drug, since they survive for as long as 20 days following their infective meals, whereas mosquitoes will not survive beyond 48 hours when deprived of food and water. Generally, in infected mosquitoes maintained on sugar solutions there is an average mortality of 30–35 per cent during the period of the experiment, and in these studies the maximum tolerated dose was considered to be that dosage in which mortality was approximately 60–70 per cent, or double that of the normal attrition. Usually, drug administration was begun 72 hours before the mosquitoes were given their infective blood meals and continued throughout the course of the infection. Later, drug schedules were varied according to the needs of an experiment.

TABLE 1

COMPARISON OF THE EFFECTS OF VARIOUS DRUGS ON THE SPOROGONOUS CYCLE OF *P. gallinaceum* IN *A. aegypti* AS DETERMINED BY SUBSEQUENT INOCULATION INTO CHICKS*

Drug	Drug conc. (grams/100 ml.)	Average per cent red cells parasitized (days after inoculation)						No. survivors
		8	10	12	14	16	18	
Quinine	.08	4.2	29.1	28.0				0/8
Quinacrine	.03	3.7	26.8	32.0	22.0			1/8
Plasmochin	.02	3.1	25.9	36.2				0/8
SN6911	.075	4.0	32.1	58	39.1			0/9
Sodium sulfadiazine	.1	0	0	0	0	0	0	8/8
Controls		2.8	31.7	30.9				1/8

* Inoculum contained one mosquito equivalent.

With this drug-diet method it has been possible to administer drugs to mosquitoes infected with *P. gallinaceum* and to test the effects of these compounds on the sporogonous cycle of the parasite. It has been found that quinine, quinacrine, plasmochin, and SN6911 administered in maximum tolerated doses have no effect on sporozoite production or sporozoite viability, and sporozoites from mosquitoes treated with these drugs, inoculated into normal chicks, produce infections indistinguishable from those produced by the inoculation of sporozoites from control mosquitoes maintained on sugar solutions alone. On the other hand, in infected mosquitoes treated with adequate concentrations of sodium sulfadiazine, oocysts fail to develop properly and sporozoites are produced only rarely, and those that are produced appear to be incapable of inducing infection when inoculated into normal chicks (Table 1).

Suspensions of mosquitoes prepared from those maintained on sugar solutions and on quinine, quinacrine, plasmochin, and SN6911 ordinarily contain two to three sporozoites per oil immersion field, whereas it may require 10 minutes examination to find a single sporozoite in suspensions prepared from mosquitoes maintained on 0.1 per cent sodium sulfadiazine. With higher concentrations of sodium sulfadiazi it becomes even more difficult to find sporozoites. Thus, dr effect can be readily discerned from microscopic examinati of suspensions prepared from drug-treated mosquitoes, a one can predict with accuracy the outcome of subsequ inoculations from suspensions prepared from drug-treated control mosquitoes.

During the course of several experiments, inoculation whole mosquitoes or of several mosquito equivalents of tho maintained on sulfadiazine levels above 0.1 per cent has fail to produce infection. Reinoculation of these negative chic has resulted in characteristic infections with high para temias. At a 0.1 per cent level oocysts are formed in lar numbers but rarely grow beyond the medial point of develo ment. With concentration of sulfadiazine increased to 0.3 p cent, there is more complete inhibition of oocyst developme and at maximum tolerated levels oocyst development appea to be more completely arrested. However, below 0.1 per cen which appears to be the critical concentration of the dru sporozoite production and viability are not too seriously a fected, and sporozoites from mosquitoes treated with low concentrations produce characteristic infections when inocu lated into normal birds (Table 2).

TABLE 2

COMPARISON OF THE RELATION OF DRUG CONCENTRATION TO DRUG ACTIVI IN *A. aegypti* INFECTED WITH *P. gallinaceum* AS DETERMINED BY SUBSEQUENT INOCULATION INTO CHICKS

Drug	Drug conc. (grams/100 ml.)	Average per cent red cells parasitized (days after inoculation)				No. survivors
		8	10	12	14	
Sodium sulfadiazine	.01	1.0	30.0	8.0		2/6
"	.05	0	1.0	8.2	23.0	2/6*
"	.1	0	0	0	0	6/6
"	.3	0	0	0	0	6/6
"	.4	0	0	0	0	6/6

* One chick remained negative.

From the known drug effects of sulfadiazine and the oth drugs cited on sporozoite-induced infections of *P. gallinaceu* it would appear, therefore, that there is a definite relatio between drug activity and effect in the sporozoite-infecte vertebrate host and drug activity in the infected inverte brate host. The analogy extends further, in that sulfadiazin has little or no effect if drug administration is delayed unt sporozoites have developed. High concentrations of dru administered over a period of several days after sporozoite have developed do not affect sporozoite viability, and spor zoites from mosquitoes so treated give rise to typical infection when inoculated into normal chicks. Furthermore, if dru administration is terminated too quickly following the in fective meal, infective sporozoites are produced even thoug development is delayed.

With this method it may become possible to evaluate drug directly in the mosquito host for their prophylactic activity against the human malarial parasite and thus to eliminate th necessity, according to present-day screening methods, o infecting large numbers of human subjects. The method ma possibly offer further promise as another means for studies o parasite metabolism and for studies on the mechanisms o drug action.

IN THE LABORATORY

Oxidation of Ascorbic Acid to Dehydroascorbic Acid

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In the Roe and Oesterling method (*2*) for the determination of ascorbic acid in plant tissue, norite is used for the oxidation of ascorbic acid to dehydroascorbic acid. The most commonly used extractant is a metaphosphoric acid solution in which norite does not oxidize ascorbic acid quantitatively to dehydroascorbic acid unless acetic or trichloroacetic acids are present in a relatively high concentration. Bromine, which has been proposed for the oxidation of ascorbic acid to dehydroascorbic acid in a metaphosphoric acid solution (*1*), is not a desirable reagent to use for this purpose because of its physical and toxic properties.

In this laboratory 2,6-dichlorobenzenoneindophenol has been used in the place of norite or bromine. The ascorbic acid is quantitatively oxidized to dehydroascorbic acid in a metaphosphoric acid solution. The color, due to the excess dye present in the sample, is destroyed when the thiourea solution is added. Not only does use of the dye permit a more rapid determination, but both the ascorbic and dehydroascorbic acids can be determined on the same acid tissue extract with a single standard curve.

Sodium 2,6-dichlorobenzenoneindophenol dye solution: Dissolve 200 mg. of the dye in 100 ml. of warm water, filter, and store in a refrigerator. Prepare freshly every two weeks.

Metaphosphoric-thiourea solution: Prepare a 20 per cent metaphosphoric acid solution in cold distilled water. Filter if necessary and store in a refrigerator. If the acid extractant used is 1, 2, 3, 4, or 5 per cent metaphosphoric acid, transfer 45, 40, 35, 30, 25 ml., respectively, of the 20 per cent metaphosphoric acid solution to a 100-ml. volumetric flask. Then add 2 grams of thiourea and distilled water. Dissolve by shaking and make up to volume. Thus, 2 ml. of the acid-thiourea solution added to 2 ml. of the extractant gives a solution containing 5 per cent metaphosphoric acid and 1 per cent thiourea.

Transfer 2 ml. of the acid tissue extract to each of three colorimetric tubes. Add one drop of the dye solution to one of these tubes. Shake tube. Some color should persist; if not, the ascorbic acid is too concentrated for obtaining best results, and the tissue extract should be diluted further with the acid extractant. Then add 2 ml. of metaphosphoric-thiourea solution to all three tubes. Reserve one tube for the blank, but to each of the other two tubes, including that to which the dye has been added, add 1 ml. of the 2,4-dinitrophenylhydrazine reagent and continue the procedure as described by Roe in his method for the determination of ascorbic acid.

A standard curve is made with known quantities of ascorbic acid in a metaphosphoric acid solution under exactly the same conditions as described for the unknown. It is important to have the same final acid concentration (5 per cent metaphosphoric acid) for all determinations if results are to be compared with a single standard curve.

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A Simple Pen for Kymograph Tracings

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In many cases a smoked kymograph drum is a decided inconvenience, and a record in ink is advantageous.

A satisfactory pen can easily be made by drawing a piece of 4-mm. glass tubing to an L-shaped capillary point and cementing[1] this into a folded piece of 16-mm. film negative. Slits made in the film enable the pen to be adjusted to any length on the lever arm. The capillary tip can be finished on very fine emery polishing paper so that it will give a smooth tracing. The pen can be refilled with a capillary tip medicine dropper while it is writing and will hold enough ink for several hours of continous tracings. When not in use, the pen is removed from the lever arm and placed in a beaker of water to prevent the ink from drying in the tip. Any fountain pen ink has been found satisfactory.

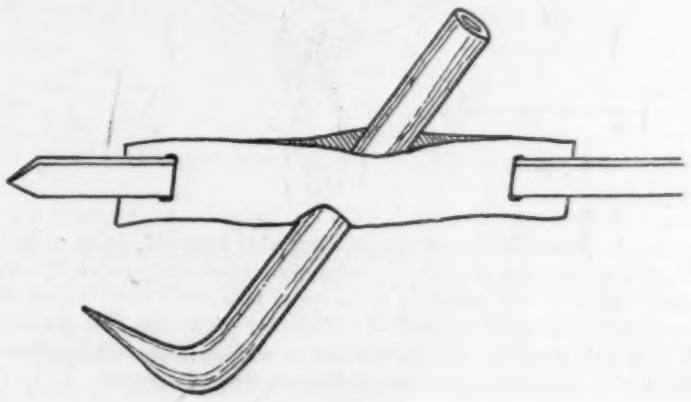

FIG. 1

The pen is not heavy enough to affect the sensitivity of respiration or activity as transmitted by a tambour and has been successfully used on signal magnets as well as in recording pressure changes in a closed system.

[1] Dupont Duco cement mixed with acetone was found most satisfactory for this.

A Simple Method for Explanting and Cultivating Early Chick Embryos *in Vitro*

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The *in vitro* technique, in spite of its usefulness in the study of many problems of embryonic development, has generally been considered far too difficult and complicated to be used in classroom experiments. This is probably true of the classical tissue culture technique with all of its special equipment and elaborate sterilization and blood-taking procedures. The following method, which has been designed for student use, dispenses with all of these but is just as effective and reliable in terms of the results it gives (Fig. 1). In the brief but complete

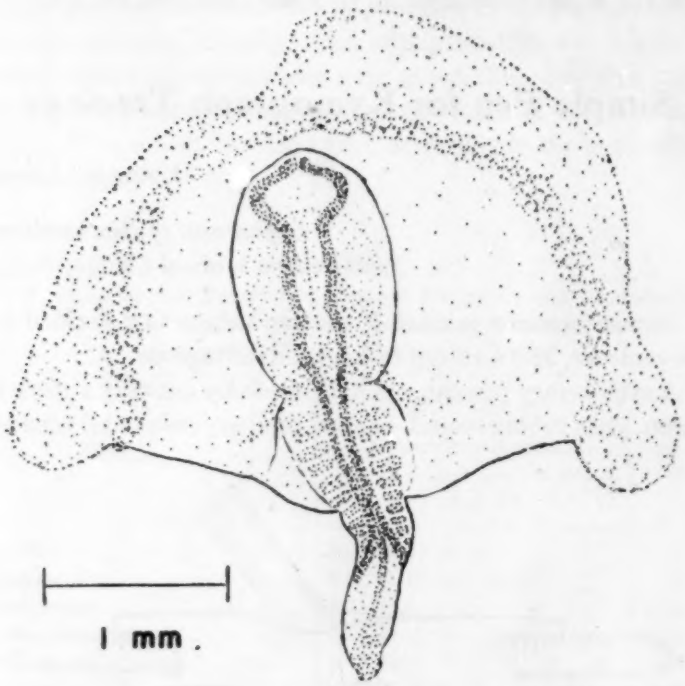

FIG. 1. Camera lucida drawing of a typical 10-hour-old, living explant of the anterior portion of a short head-process blastoderm which was transected about 0.4 mm. posterior to the node. This result was obtained after carrying out verbatim the method outlined for saline-agar-albumen media. With a little practice, the student can accumulate many similar cases of beautifully symmetrical and essentially normal morphogenesis.

procedure outlined below, no sterilization of equipment or solutions is necessary because of the bacteriolytic property of egg albumen used in the culture medium. Tap water may be used in place of distilled water. (When synthetic media, yolk-extract media, etc. containing no egg albumen are used, all glassware and instruments are dry sterilized and solutions are autoclaved.)

Equipment. The following ordinary laboratory equipment is all that is necessary: 1 dozen Petri dishes (4 inches in diameter), 1 dozen watch crystals (2 inches in diameter), absorbent cotton (ca. 40 grams), 1 finger bowl (4½ inches in diameter), 2 Erlenmeyer flasks (ca. 500 cc.), 1 Erlenmeyer flask (ca. 125 cc.), 1 graduated cylinder (100 cc.), 1 wide-mouthed pipette (inside diameter, 3–4 mm.), 1 fine pipette (inside mouth diameter, ca. 1 mm.), 1 student dissecting set (2 needles, 1 pair of scissors, 1 pair of forceps), and 1 dissecting microscope.

Preparation of equipment. The glassware and dissecting instruments are thoroughly washed, rinsed in hot, running, tap water, and set aside to dry on a clean towel. The culture dishes (which have a large, humid, air space) are like those used by Fell and Robison (*1*). These are assembled by placing a moist cotton ring in each Petri dish, placing a watch crystal (concave side up) on this, and replacing the cover of the dish.

Preparation of culture medium. The final medium consists of two components which are made up separately and combined just before the medium is placed in the culture dishes. *Part I* (Ringer-albumen): The egg white from one unincubated egg (after separation from the yolk in the usual fashion) is added to 50 cc. of ordinary chick Ringer solution (0.9 per cent NaCl, 0.042 per cent KCl, 0.024 per cent $CaCl_2$) contained in a 500-cc. flask. The flask is stoppered and shaken vigorously for about 1 minute. *Part II* (Ringer-agar): 0.13–0.15 gram of agar (U.S.P. XI) is placed in the small flask along with 30 cc. of Ringer solution. The Ringer-agar is carefully brought to a boil over a Bunsen burner. A small flame is used, and the flask is frequently agitated to prevent the agar from sticking.

When the Ringer-agar has cooled down to about 40°–45° C., 20 cc. of the Ringer-albumen component (exclusive of the foamy portion) is added, and the flask is gently shaken to mix the two. Approximately 2 cc. of the medium is poured out into each watch crystal. The medium is allowed to gel (ca. 30 minutes–1 hour) before the Petri dishes are moved.

Operative procedure. Eggs containing the embryos to be cultivated[1] are opened into a finger bowl containing about 100 cc. of Ringer solution. The yolk is held steady with the forceps while a cut (ca. ¼ inch from the border of the blastoderm) is made through the vitelline membrane with the scissors and carried all the way around the blastoderm. The vitelline membrane with the blastoderm adhering to its underside is grasped at the cut edge and gently rolled back from the yolk. The blastoderm is then freed from the membrane by gently working a dissecting needle around its border (a fairly blunt needle is best and can be used to push or roll the blastoderm away from the membrane). It is then transferred in the wide-mouthed pipette to a Petri dish containing about 20 cc. of Ringer solution. Under a dissecting microscope, most or all of the yolky opaque area is trimmed off with the dissecting needles. When finer operations are performed on the embryo, it is important to use freshly sharpened needles.

Culture procedure. The embryo or parts of it to be cultivated are transferred in a wide-mouthed pipette to the surface of the culture medium, oriented as desired, and flattened out by gently sucking away the excess saline with a fine pipette. The blastoderm may be marked with carbon powder if desired (*2*). The Petri dish is covered and placed in the incubator.

With minor modifications,[2] the basic procedure outlined above is being used in the study of some of the problems of early embryonic nutrition, morphogenesis, localization of organ-forming areas, etc. The results of some of these studies will be reported in the near future.

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[1] Incubation at ca. 38° C. for 20–25 hours will furnish definitive primitive streak, head-process, head-fold, and early somite blastoderms. A simple incubator made out of a desk lamp and a cardboard carton can be used for the eggs as well as the cultures.

[2] For example, when glucose (100–800 mg. per cent, added to the Ringer-agar solution) is substituted for the albumen, some interesting effects on development can be observed.

An Interesting Case of Water Undercooling

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"If a pure liquid is carefully protected from mechanical disturbances, it may be cooled below the temperature at which it normally solidifies. Thus, water may be cooled to −10° C. or lower without becoming ice. The liquid at such a temperature is in a state of unstable equilibrium and will immediately solidify if disturbed or if a crystal of the solid is dropped into it" [1]. Some such statement appears in physics texts when the subject of undercooling of a liquid is discussed. The difficulties of obtaining a successful mass demonstration of the undercooling of water are well known.

In investigations of the operating characteristics of home freezers in this Bureau, the tests include the freezing of a volume-capacity load with water as the test material in pint, paraffined, nested, cardboard containers with disc lids. Copper-constantan thermocouples are used with recording potentiometers. Since each instrument records its series of 16 points in approximately one minute, the temperature at each location can be followed quite closely.

Temperatures are taken in enough cartons, distributed throughout the freezing load, to be sure that they are obtained in the first and last cartons to freeze. In the case reported here, temperatures were taken in 7 of the 67 cartons comprising the full load. Undercooling occurred in 5 of these 7 cartons. If the phenomenon took place in the same proportion for all of the cartons, 48 of them undercooled.

The requirements generally stressed in textbooks for the phenomenon of undercooling are purity of liquid, absence of foreign substances, and absence of mechanical disturbance. Under the conditions in which the freezing tests are conducted in our laboratories, undercooling of water might be expected to be a rare occurrence. Actually, however, it is the rare occurrence when one or more of the cartons in which thermocouples are placed in a freezing load do not show undercooling.

In this particular part of the experimental work, there is no necessity for having the materials scrupulously clean. The thermocouples are either new, no attempt having been made to remove the soldering flux completely, or have been kept loose in a drawer since their previous use. More often than not, the cartons have been reparaffined one or more times if, in previous use, freezing cracked the seams. The water used is the ordinary distilled water of the laboratory, placed in the cartons the day before and allowed to stand uncovered at least 17 hours before the start of the test.

The test is begun at the start of an "on" period of a cycle. Hence, the cartons are subjected to continuous vibration if the compressor stays in operation until the end of the "freezing period," *i.e.* until the temperature in the last carton reaches 10° F. If the compressor cycles during that time, the cartons are subjected to the sudden jarrings at the start of the "on" periods and vibration during the running parts of the cycles.

The freezing compartment of the freezer being tested in the case discussed here was directly above the compressor, thus receiving the full impact of the starting of each period of operation. To check the magnitude of the agitation during the cycling of the compressor unit, at the conclusion of the test some uncovered cartons were distributed throughout a load in the freezing compartment. Standing waves of quite large amplitude were observed in each such carton, indicating that rather forceful disturbance was present while the compressor was in operation. During the test the compressor unit ran continuously for about 3 hours after the load was placed and then cycled approximately 260 times during the remaining 93 hours of the test. Some one or more of the cartons was in an undercooled state during 60 of these hours, during which time the compressor cycled 160 times. These figures do not support part of the quotation, viz., "The liquid at such a temperature is in a state of unstable equilibrium and will immediately solidify if disturbed. . . ."

Fig. 1 gives the temperature-time relationships in 6 of the 7 cartons in which temperatures were taken. Curve F is typical of those for a liquid passing through its solidification tempera-

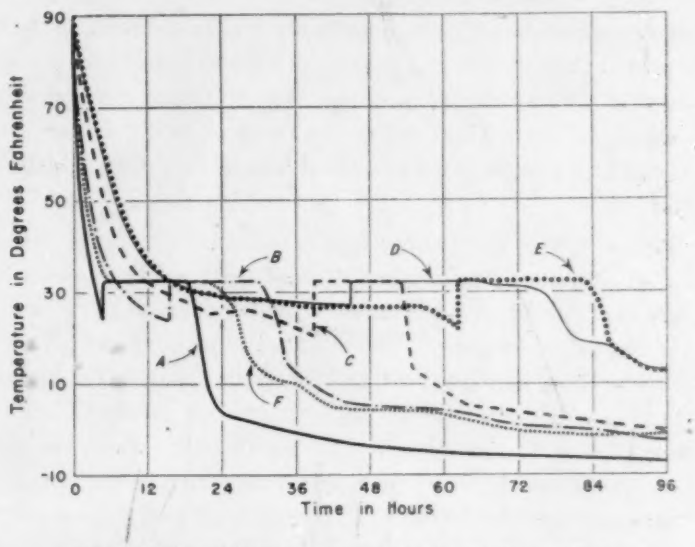

FIG. 1. Undercooling of water during home-freezer test.

ture without undercooling. Curves A and B are typical of the usual temperature-time relationships observed when undercooling does occur. Curves C, D, and E are seemingly atypical, but show the relationships when undercooling is prolonged.

Rapidity of cooling to and through 32° F. was not the deciding factor as to whether or not undercooling occurred. Curve A passes rapidly through the solidification temperature of 32° F., Curve E very slowly, and yet each exhibits undercooling. Curve F, representing a carton in which undercooling did not take place, came to the solidification temperature at a speed intermediate to these two. The temperature in the 7th carton (curve not shown) dropped to 32° F. more rapidly than any of the 6 for which curves are shown, and the water did not undercool.

An interesting feature shown by the graph is that, during the 9-hour period from 30 to 39 hours, the water in none of the cartons was at 32° F.; in three cartons the water had completely solidified, and in the other three it was in an undercooled state. Apparently the phenomenon of undercooling of water which is thought of as occurring only under nearly ideal conditions actually takes place under conditions far from ideal and does so to such an extent as to make questionable the accepted restrictions on the conditions required for its occurrence.

Reference

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Book Reviews

Fundamentals of earth science. Henry Dewey Thompson. New York-London: D. Appleton-Century, 1947. Pp. xiii + 461. (Illustrated.) $3.75.

In the preface it is stated that the book is written with a twofold purpose. The first objective, according to the author is "to present a well-balanced, one semester survey of earth science which will give the college student who takes only one course a rather complete elementary picture of the earth." The second objective is "to provide a basic text for beginning students in either geology or geography." Dr. Thompson has achieved both purposes admirably.

The student who does not go on for additional work in these fields will benefit culturally by acquiring an intelligent appreciation of scenery and a knowledge of the physical environment in which he lives. The student who desires to dig deeper into geology and geography has a good foundation upon which to build.

The author of any survey text is faced with the problem of how much material he can include and still keep the volume to a size that can be covered in one semester of study. The material covered is extensive, carefully selected, and includes all branches of earth science. About 25 pages are devoted to historical geology. Generally, the material is presented with thoroughness. In the opinion of the reviewer, however, the role of chemical weathering in semiarid and arid regions does not receive enough emphasis, and the part played by chemical weathering in *exfoliation* should be mentioned. For teaching purposes it would be of greater service if the cloud families were included.

Although the reproduction of several of the illustrations is not very clear, the book is amply illustrated and the author is to be commended for his explanation and use of aerial photographs. The choice of words, the clarity, and the ease with which the book can be read are outstanding features.

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Quantitative clinical chemistry: interpretations. (Vol. I.) (2nd ed.) John P. Peters and Donald D. Van Slyke. Baltimore: Williams & Wilkins, 1946. Pp. vii + 1041. (Illustrated.) $7.00.

The first edition of this text by these two outstanding authorities in the fields of clinical chemistry and clinical medicine quickly gained recognition as the authoritative work in this field. The chemical and physiological facts and their interpretation in disease were presented on the basis of an exhaustive review of the literature, with citation of references. In the 15 years which have elapsed since the first edition so many advances have been made that the authors felt it necessary to rewrite, not re-edit, the first edition.

In Volume I of this second edition the material presented in the first half of the original volume on interpretations has been reclassified and expanded to about double the original size. The new Volume I consists of four parts, Energy Metabolism, Carbohydrates, Lipids, and Protein Metabolism, presented in 13 chapters. Two of the chapters under lipids are new, viz., steroid hormones and fat-soluble vitamins.

The authors have done an excellent job of reviewing the enormous literature (4,600 references) and presenting the pertinent facts in logical order. Furthermore, the style is such that the experimental data covering the important observations are connected to make easy and stimulating reading. The material has been presented critically on the basis of the authors' intimate and expert knowledge of the field. If the reader should disagree with interpretations or desire fuller information, the unusually complete bibliography will enable him to consult the original papers. The text is unusually free from typographical and other errors. It should be available to all students and teachers of biochemistry and is an essential reference book for clinical chemists and up-to-date internists and pediatricians.

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Essentials of endocrinology. (2nd ed.) Arthur Grollman. Philadelphia: J. B. Lippincott, 1947. Pp. xxiii + 644. (Illustrated.) $10.00.

The decline of interest in endocrine research caused by the late war would seem to create a propitious moment for the crystallization of available information in the form of a book such as that being reviewed. However, the wide ramifications of endocrinology and the many fields of basic science which it involves make the writing of such a book by a single individual a tremendous task indeed.

In this attempt, the anatomy, physiology, pharmacology, and pathology of the ductless glands are discussed as well as endocrine diseases and their treatment. The application of the findings of basic endocrine research to clinical medicine is viewed with a refreshing conservatism. Portions dealing with clinical problems and pathology are well illustrated. Less can be said for some of the illustrative material dealing with the anatomy of the glands. Inaccurate or illogical statements, although occasionally found, seem to be remarkably few in number. Generalizations drawn from scattered research findings in such an immature field are certain to find ready opposition. This is true of many of the author's statements. If intended for use as a text, reorganization of some of the material to give greater conciseness of presentation would be desirable.

This book is the best of its kind available. Whatever major criticism might be raised against it would arise largely from the problems faced by any one individual who seeks to master all phases of endocrinology so that he may acquire sufficient background to authoritatively select and present information with the proper perspective.

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